DEVELOPMENT OF A BIOHERBICIDE FOR THE CONTROL OF PURPLE NUTSEDGE

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The possibility to utilize an indigenous fungus to control purple nutsedge (*Cyperus rotundus* L.) was investigated in this study. A fungal pathogen, *Dactylaria higginsii*, was determined to have the potential to control purple nutsedge. When applied as a postemergence foliar spray, the fungus inflicted a high percentage of mortality of plants in a number of *Cyperus* spp. In contrast, *D. higginsii* was not pathogenic to several beneficial plants and economically important crops species tested. The level of disease severity on purple nutsedge was linearly related to the conidial concentration of *D. higginsii* between 10^2 and 10^6 conidia per milliliter of the inoculum suspension. The most susceptible age of purple nutsedge plants was the 4- to 6-leaf stage, which is within the application time of conventional postemergence chemical herbicides. The optimal

dew-period temperature was 20°C to 30°C, which is also within the temperature range during the cropping season. *Dactylaria higginsii* required a minimum of 12 h of dew to infect purple nutsedge. Such long dew duration could be a constraint to the use of this bioherbicide in the field. However, this constraint may be overcome by early application of *D. higginsii* during the cropping season, when the temperature and dew-period are not limiting. Alternatively, the dew requirement may be bypassed by adding suitable amendments to the inoculum when it is applied later in the growing season. Studies on the effect of *D. higginsii* on purple nutsedge interference with two crops, tomato and pepper, confirmed the ability of this pathogen to reduce purple nutsedge growth components, which can translate into a suppression of purple nutsedge interference and an increase in crop yields. In field trials, a single application of *D. higginsii* at 10^6 conidia per milliliter did not effectively reduce the growth of purple nutsedge plants due to the low level of secondary disease spread and inadequate disease to curtail the fast regeneration of purple nutsedge. However, the level of purple nutsedge control could be increased significantly by three applications of *D. higginsii*. Thus, the potential of *D. higginsii* to be used as a bioherbicide to control purple nutsedge was demonstrated.

CHAPTER 1
LITERATURE REVIEW

The deleterious impact of weeds on humankind has been recognized since the beginning of agriculture. Despite the use of modern weed-control technology and immeasurable amounts of human labor, weeds are still perceived by farmers and advocates of sustainable agriculture to be the major deterrent to the development of new crop-production systems. Weeds cause substantial yield reductions of crops by competition and allelopathic effects. They also reduce food, feed, and fiber quality. In the United States, more than $6 billion is spent annually on herbicides, tillage, and cultivation to control weeds in croplands (Chandler, 1991), yet the annual losses due to weed infestation currently exceed $4 billion (Bridges, 1992; Bridges and Anderson, 1992; and Chandler et al., 1985). In many developing countries, hand weeding consumes most of the labor involved in crop production (Akobundu, 1991). Akobundu (1987) estimated that weeds reduce crop yields by 5% in the most developed countries, 10% in the developing countries, and 25% in the least developed countries.

Over the last four decades, chemical herbicides have dominated weed-management strategies in the developing countries (Wyse, 1992; Abernathy and Bridges, 1994). They have been perceived as a modern miracle and a scientific breakthrough in weed control (Holt, 1992). In the United States, herbicides have become the major component of weed management and have accounted for nearly 60% of the total amount

of all agricultural pesticides applied (Aspelin, 1994). In many developing countries, an increase in crop production has been viewed as dependent on an increase in the use of agrochemicals (Conway and Barbier, 1990). The increase in reliance on herbicides has been accompanied by marked improvements in crop productivity and efficiency in farm labor. One such example is the ability of the U.S. farmers to produce surplus food for millions of people worldwide despite only 2% of the American population being engaged in agriculture (Peeples, 1994).

Unfortunately, over-reliance on chemical herbicides has caused farmers to face increasingly intractable weed problems with few options for chemical control. Herbicide resistance, which has been noted in more than 100 weed species and has been calculated to evolve under field conditions in 4 to 5 years (Holt, 1992), is now appearing in new species at a rate equal to that observed for insecticide resistance in arthropod pests (Holt and LeBaron, 1990). Growing numbers of government regulations and restrictions, and increasing costs of research, development, and registration are expected to reduce the availability of older herbicides and the rates of introduction of new herbicides (Holt and LeBaron, 1990). The general fear of chemicals by the public, combined with the environmental movement that is sweeping many parts of the globe, has led to unfounded accusations by various anti-pesticide groups that herbicides are a major threat to public health and environment (Abdallah and Libby, 1987; Halberg, 1987; Kearny et al., 1988). This has led to the banning of many present-day products in many parts of the world and the increase of more stringent and costly regulations in most countries.

In addition to shifts in genotypic frequencies within weed populations, heavy

reliance on herbicides has, in some cases, shifted the composition of weed communities toward species that are more difficult to control. One such example is purple nutsedge (*Cyprus rotundus* L.). According to Bendixen and Stoube (1977), purple nutsedge was not a problem weed where quackgrass (*Agropyron repens* (L.) Beauv.), johnsongrass (*Sorghum halepense* (L.) Pers.), bermudagrass (*Cynodon dactylon* (L.) Pers.), and broadleaf weeds were established but was a problem in cultivated agriculture where the competitive pressure from other weeds was controlled by herbicides. Once purple nutsedge becomes established in an area, it has the capability to colonize large, disturbed areas (i.e., agricultural areas) at an alarming rate. The reason for this phenomenon is the weed's competitiveness and its ability to propagate prolifically through tubers and rhizomes.

Purple nutsedge is a serious weed worldwide including the United States (Bendixen and Nandihalli, 1987; Doll, 1983; Keeley, 1987). It has been called the world's worst weed because it is a serious competitor with more crops in more countries of the world than any other weed (Holm et al., 1977). It is a perennial weed that superficially resembles grasses. Although it is native to the Indian subcontinent (Ranade and Burns, 1925), it can be found in more than 90 countries throughout the tropical and warmer temperate regions of the world. Its distribution in the United States is confined to the southeastern region of the continent, California, and Hawaii.

Purple nutsedge grows well in almost every soil type and over a wide range of soil moisture, pH, and elevations (Holm et al., 1977). However, it does not tolerate shade, cold temperature, and drought. The success of this troublesome weed and its propensity

to infest large areas of croplands are mostly due to its ability to survive and reproduce from tubers during adverse conditions.

Genetic analysis done on various localized populations of purple nutsedge has shown that genetic diversity of this weed was limited in comparison to its related species, yellow nutsedge (*Cyperus esculentus* L.), which showed extreme variability among the biotypes examined (Holt, 1994; Horak and Holt, 1986). This finding was confirmed by Okoli et al. (1997), who demonstrated through randomly amplified polymorphic DNA (RAPD) analysis that collections of purple nutsedge showed very limited intraspecifc variation, with most of the collections showing no differences. This is indicative of the ability of purple nutsedge to form large regional clones, and that asexual reproduction is the predominant means of propagation.

Even though purple nutsedge is a short-statured plant, it has the ability to exert early competition on crops. This is another reason for its classification as the world's worst weed. In addition to its ability to exert early competition, purple nutsedge produces an allelopathic substance in the underground parts of the plants which has an inhibitory effect on root and shoot development of many crop plants (Leela, 1995; Horowitz and Friedman, 1971; Singh 1968).

Biology and Life History

Purple nutsedge belongs to the family Cyperaceae (Ranade and Burns, 1925). It resembles a grass in growth habit, but produces above-ground shoots that initially consist of a triangular stem-like fascicle of leaves which later develops into a solid triangular

stem or culm that extends through the center of the fascicle and bears a seed head at the apex. Purple nutsedge has a reddish-brown or purplish-brown inflorescence. Leaves, which are dark green with pointed (boat-shaped) leaf tips, are V-shaped in cross section and are arranged in a set of three at the base. The upper leaf surface is predominantly composed of waxy cuticle and is devoid of stomatal openings. The lower surface is thinly cutinized and has many parallel rows of stomata.

Purple nutsedge possesses the highly efficient C_4 dicarboxylic acid photosynthetic pathway (Wills, 1987). Plants with the C_4 pathway are efficient in assimilating CO_2 at higher temperatures and light intensities (Black et al., 1969; Downton and Tregunna, 1968). Black et al. (1969) noted that species which fix CO_2 at high rates under conditions of increased temperature and light intensity, coupled with characteristics such as rhizomatous spread, have the potential to be serious weed pests.

Purple nutsedge is propagated predominantly by tubers and forms an interconnected network of rhizomes, tubers, and basal bulbs. The rhizomes spread in all directions and exposure of the rhizomes to fluctuations in light and temperature stimulate them to form basal bulbs. Ninety percent of all tubers are formed in the upper 45 cm of soil (Smith and Fick, 1937; Tripathi, 1969; Thumbleson and Kommedahl, 1961). In most soils, more than 80% of the tubers are in the upper 15 cm (Stoller and Sweet, 1987). Tubers make up the greatest portion of the total plant weight and the bulk of the underground weight (Horowitz, 1972). Tuber production by purple nutsedge is rapid. In Georgia, the rate of tuber production at the end of the first summer was 63 per each tuber planted at 30-cm distance and 913 tubers at 90-cm intervals.

The tubers sprout between temperatures of 10°C and 45°C with 35°C being the optimum (Bhardwaj and Verma, 1968). However, the tubers do not spout uniformly. For example, in Jamaica, 35% of tuber sprouting occurred in September, whereas 83% of tubers sprouted in June (Hammerton, 1975). The differential tuber sprouting was due to tuber dormancy, which was related to the presence of salicylic acid, abscisic acid, and an inhibitor B (Teo et al., 1973; 1974). Most purple nutsedge tubers remain dormant as long as they are connected to the mother plant. The dormancy, however, is lost once the tuber is severed from the chain. Hence, cultivation followed by favorable conditions, will cause an increase in the number of emerging shoots. Purple nutsedge has two forms of apical dominance: apical dominance exercised by the terminal tuber over the other tubers in the chain and apical dominance exercised by the apical bud of a tuber over the other buds (Marcado, 1979). Zandstra et al. (1974) found that the degree of apical dominance is influenced by the age of the tuber chain; younger tubers were less dormant than older chains.

Reproduction

Sexual. Purple nutsedge is capable of producing up to 60 inflorescences/m^2, and each inflorescence can produce up to 220 seeds. This is equal to 131 million seeds per hectare (Ranade and Burns, 1925). Such seed yields can be produced up to three times per year. Seeds have been reported to be produced readily in young, vigorously growing populations, but are often absent in most populations (Bell et al., 1962; Holm et al., 1977). Although seeds of purple nutsedge have been reported to be viable, seed

germination is seldom higher than 5% in nature without special treatments (Holm et al., 1977). Many researchers who have investigated nutsedge propagation feel that seeds are not an important source of purple nutsedge dissemination even though mature seedlings can be derived from seeds (Justice and Whitehead, 1946; Smith and Fick, 1937; Thullen and Keeley, 1975).

Asexual. Asexual reproduction through tubers and bulbs is the major means of propagation for purple nutsedge, and it has been responsible for the worldwide dissemination of the species. Tubers of purple nutsedge showed a high capacity to sprout throughout the year (Horowitz, 1972), with the greatest number of sprouts produced during winter and spring, and the fewest during late summer to early fall (Taylorson, 1967). Dormancy is thought to be due to chemical inhibitors (see above), as washing tubers in cold water increased the number of tubers sprouting (Doll, 1983). Dormancy is broken in an acropetal order, starting from the oldest bud (Doll, 1983). The number of buds that break dormancy is dependent upon how soon sprouts are removed from the tubers (Bendixen, 1973). Soil temperature can also affect the number of tubers that sprout. When temperature was raised from 10°C to 39°C, the number of purple nutsedge tubers that sprouted rose from 0% to 59-100% (Horowitz, 1972). Neither multiple sprouting nor readiness to sprout was affected by tuber weight (Stoller et al., 1972; Thullen and Keeley, 1975), but seedling size and vigor were highly correlated with tuber weight and sizes (Thullen and Keeley, 1975).

Multiple sprouting of larger tubers (>0.75 g) have been reported (Santos et al., 1997a), and in some instances tubers have been reported to sprout up to six times

(Bendixen, 1973). Work by Stoller et al. (1972) showed that 60% of a tuber dry weight in carbohydrate, oil, starch, and protein reserves is used by the first sprout. Six to 18% of these reserves are utilized by the second sprout and 2 to 10% by the third sprout. For this reason, the proportion of purple nutsedge tubers that regenerate new growth decreases after each sprout is produced (Stoller et al., 1972).

Economic Importance of Purple Nutsedge

Purple nutsedge is not an important forage plant because the plant is less nutritious and the leaves become very fibrous rapidly. It is not a preferred forage plant for most animals except for the wild boar that prefers foraging on the tubers rather than on the foliage (Bendixen and Nandahili, 1987). Purple nutsedge tubers contain sucrose, glucose, and fructose (Wills, 1972; Wills and Briscoe, 1970); however, they are not readily used as food because of their undesirable flavor.

Purple nutsedge poses a significant problem in field crops (Wax, 1975), horticultural crops (Sweet, 1975), and turfgrass (Tweedy et al., 1975). The critical period of competition between purple nutsedge and crops varies. Crops that are less competitive have relatively longer critical periods accompanied by heavy yield losses than fast-growing crop species. The critical period of slow-growing crops may vary from 3 to 13 weeks while this may last for only 3 to 5 weeks for the fast-growing species (William and Warren, 1975). Under humid climate, purple nutsedge plants are able to mobilize and store 815 kg of ammonium sulfate, 320 kg of potash, and 200 kg of super phosphate per ha (Rochescouste, 1956). Bhardwaj and Verma (1968) reported that purple nutsedge is

capable of removing 94.6 kg of N, 11.6 kg of P_2O_5, and 96.4 kg of K_2O per ha and more than 50% of these nutrients were stored in the tubers.

In Brazil, the presence of 600 to 1,600 purple nutsedge plant/m^2 reduced crop yields as follows: garlic (*Allium sativum* L.) 89%, okra (*Hibiscus esculentus* L.) 62% , carrot (*Daucus carota* L.) 39 to 50%, green bean (*Phaseolus vulgaris* L.) 41%, cucumber (*Cucumis sativus* L.) 43%, and tomato (*Lycopersicon esculentum* Mill.) 53% (William and Warren, 1975). In El Salvador, 700 purple nutsedge plants/m^2 reduced corn (*Zea mays* L.) yield by 43% (Chase and Appleby, 1979). In the Philippines, an increase in density of purple nutsedge reduced the yield of rice (*Oryza sativa* L.) (Okafor and De Datta, 1976). As a result of purple nutsedge interference, yield reductions of 32, 36, and 39% occurred in rice at 0, 40, and 120 kg/ha of N fertilizer, respectively.

Chapman (1966) indicated that purple nutsedge competes with sugarcane for moisture and that depletion of moisture at stooling time of sugarcane can cause fewer canes to be produced and lower the yield. As little as 40 to 100 plants/m^2 can cause up to 45% reduction in sugarcane yield (Holm et al., 1977; Keeley, 1977; Stoller and Sweet, 1987). Okafor and De Datta (1976) found that competition by purple nutsedge for soil moisture contributed to poor rice yield, especially as the nitrogen rates were increased. When purple nutsedge was grown in association with crops, it reduced N and K content in cotton shoots (Guantes and Mercado, 1975) and N and P content in young, growing wheat (Soni and Ambasht, 1977). In recent years, the degree of competition from purple nutsedge has increased dramatically due to the lack of crop rotation and the lessened use of hand cultivation (Bendixen, 1973; Glaze, 1987; Holm et al., 1991). The use of

selective herbicides, which do not control purple nutsedge but control much of the annual and less severe perennial weeds, has increased the potential of purple nutsedge to become the most frequently reported weed in agricultural crops (Horowitz, 1972, 1992; Bendixen and Stoube, 1977).

Control of Purple Nutsedge

Cultural control. Purple nutsedge is one of the most difficult weeds to control. A reason for this is that it reproduces through vegetative tubers and rhizomes that remain dormant when the environmental conditions are unfavorable. Unless these tubers are killed or their production reduced, control measures provide only a temporary solution. Increases in annual weed control and improved crop-growing practices (increases in production inputs) often exacerbate the nutsedge problem.

Glaze (1985, 1987) and Doll (1983) have discussed several cultural and mechanical methods to control purple nutsedge. Purple nutsedge thrives under intense cultivation. Therefore, timing of cultivation can help to force the tubers to sprout before crop planting, thereby allowing the use of desiccation, cold damage (Glaze, 1987; Stoller and Sweet, 1987), or preplant postemergent herbicides as a means of control (Doll, 1983). The effect of removing the shoots from growing purple nutsedge has been studied by Santos et al. (1997a), Marambe, (1996), and Komai and Ueki (1982). These researchers found that shoot removal is effective in reducing the biomass of purple nutsedge only if it is done early in the season and frequently; however, Santos et al. (1997a) did not observed total tuber depletions with plants having tubers larger than 0.75 g. They

postulated that the removal of shoots from larger tubers removes apical dominance and that larger tubers have more sprouting buds; thus the plants are able to regenerate more shoots. They suggested that a frequent mowing program is a valuable practice in reducing tuberization from larger tubers and is necessary for effective purple nutsedge management.

Tuber longevity is dependent on the depth of the tuber in the soil, soil moisture, and soil and air temperature (Doll, 1983). As such, methods that would subject the tubers to certain levels of these factors may be effective to control the weed. For example, preplant tillage can move tubers to the soil surface where they are subjected to desiccation and cold damage. Tillage operations that exposed tubers to the soil surface for at least 2 days decreased sprouting by up to 93% in yellow nutsedge (Thumbelson and Kommedahl, 1961). Purple nutsedge tubers can survive storage in soil containing 3 to 10% moisture for 15 days (Baker, 1964). Thomas (1969), however, found that duration of desiccation did not influence tuber survival in purple nutsedge, except at lower temperatures (e.g., 4°C). Purple nutsedge is almost exclusively restricted to areas where the average minimum air temperature for January is higher than -1.1°C (Stoller, 1973). Therefore, practices that would bring tubers up to the soil surface could increase the number of tubers killed.

Nonetheless, deep ploughing is seldom effective as shoots can emerge from tubers buried as deep as 88.9 cm (Loustalot et al., 1954), a depth that is not usually reached by conventional tillage methods. In fact, as sprouting is regulated by apical dominance, which could be overcome by segmenting tuber chains, tillage usually increases the

number of nutsedge shoots produced, worsening the weed problem (Baker, 1964, Horowitz, 1972). Fragmented tubers will actually produce more shoots and more top growth than whole tubers on a per-tuber basis (Baker, 1964). Although short intervals between cultivation disrupt growing tubers more often, the practice actually increases the number of sprouts produced and increases the lifespan of tubers (Thullen and Keeley, 1975). Purple nutsedge can be controlled mechanically by tilling every 3 weeks for a 2-year period, sometimes followed by clean-cultivation in the third year (Doll, 1983; William and Bendixen, 1987). When tillage is effective, it can take at least 2 years of tilling of the field every two or three weeks to reduce the nutsedge population to manageable levels (Glaze, 1985).

Fallowing for 4 years has decreased tuber yield from 912 to 7 tubers per square foot and tuber viability from 72 to 28% (Thumbelson and Kommedahl, 1961). Not many farmers can afford to leave land fallow for such a long period, however. Reduction in the length of the growing season by delayed planting or early harvest will also reduce the growth (dry-matter accumulation) and tuber production of purple nutsedge (Jordan-Molero and Stoller, 1978), but may cut deeply into the farmer's profits.

The competition by nutsedge can be radically decreased by supplementing the existing control measures with cultural practices that encourage crop shading (Glaze, 1987; Holm et al., 1991; Shetty et al., 1982). Intense shading and high crop densities can help to reduce the nutsedge populations, and eventually control the weed (Glaze, 1985, 1987; William and Bendixen, 1987; Santos et al., 1997b). Although Keeley and Thullen (1978) noted that nutsedge can be controlled more effectively with intense shading, but

shading alone would not eradicate the weed. Petterson (1981) confirmed these results, and Shetty et al. (1982) and Santos et al. (1997b) found similar results with yellow nutsedge.

Practices that shorten the time taken for the crop to produce a complete canopy that can shade nutsedge will reduce the impact of nutsedge on crop plants and increase crop yield (Glaze, 1987). Such practices might include increased seeding rates, decreased row spacing, and use of crop species that form canopies quickly. Plants that can effectively reduce nutsedge populations by developing dense canopies include sweet potato (*Ipomoea batatas* Lam.), soybean (*Glycine max* [L.] Merr.), and cotton (*Gossypium hirsutum* L.) (William, 1976). A denser crop will also increase the moisture under the canopy and create a microenvironment that is conducive to foliar pathogens of nutsedge. Also, the high crop density will favor crop competition and reduce weed growth.

Mulch is not effective for purple nutsedge control as nutsedge can penetrate both organic and physical mulches (William, 1976). In some cases, mulch increased nutsedge growth by reducing competition from other weeds that could not penetrate the mulches (William, 1976). Keeley and Thullen (1978) studied the effect of shading on purple and yellow nutsedges with plastic shade cloth. They found that a 10-fold decrease in average shoot and tuber number and total dry-matter accumulation occurred with 30% shade. Even though total dry-matter production decreased significantly, dry weight of individual tubers was not affected. Tuber production by purple nutsedge was not eliminated.

Chemical control. Chemical control of purple nutsedge is generally difficult

because of the complexity of the leaf morphology. Many crop-selective herbicides have failed to provide adequate control of this weed. With some notable exceptions, herbicides are often only marginally effective in controlling purple nutsedge because they must either penetrate a thick waxy upper leaf cuticle or enter through the stomata or cutinized cells of the lower leaf surface to the vascular system and then to the dormant meristems of the tubers (Holm et al., 1991; Stoller et al., 1972; Wills, 1987; Wills and Briscoe, 1970). Many nonselective herbicides, such as glyphosate [N-(phosphonomethyl)glycine], will work on an annual basis on nutsedge, but the rhizomes and tubers are unaffected even after the deterioration of shoot and fleshy root matter. This phenomenon is evidenced by continued apical dominance of one tuber over the others in the chain (Holm et al., 1991). However, the intact vascular system does not increase the possibility of control by systemic herbicides, due to the dormancy of the tuber meristems. Before 1987, there were only a few chemical herbicides that were marginally effective in controlling purple nutsedge. Moreover, these herbicides have had practical problems in their usage, such as translocation to the site of action, inconsistent control when applied to different growth stages, and problems with environmental conditions during or following application (Pereira et al., 1987). Since 1987, several herbicides in the sulfonylurea and imidazolinone families have been released for use in several agronomic crops. Chlorimuron [2-(((((4-chloro-6-methoxy 2-pyrimidinyl) amino) carboxyl) amino) sulfonyl) benzoic acid], nicosulfuron [2-(((((4,6-dimethoxy-2-pyrimidinyl) amino) carbonyl) amino) sulfonyl) benzoic acid], and primisulfuron [2-(((4-6-bis(difluoromethyl)-2-pyrimidinyl) amino) sulfonyl) benzoic acid] as well as imazaquin

[2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3 quinoline carboxylic acid] and imazethapyr [2-(4,5-dihydro-4-methyl-4-(1-methyl)-5-oxo-1H-imidazole-2-yl)-5-ethyl-3-pyridinecarboxylic acid] have exhibited activity against purple nutsedge in peanuts, corn, and some other cropping situations (Derr and Wilcut, 1993; Grichar et al., 1992; Richburg et al., 1993a; 1993b; Wehtje et al., 1993). Reddy and Bendixen (1988) studied the activity of foliar-applied chlorimuron and found good levels of control of nutsedge one month after application of the highest rate, but they also found that chlorimuron was readily absorbed and translocated, and persisted in the plants for relatively long periods of time. Imazameth {(±)-2-[4,5-dihydroxy-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5methyl -3-pyridinecarboxylic acid}, which is in the imidazolinone family, when applied as foliar postemergence spray at 0.063 kg/ha to peanut in Florida and Georgia provided 99% control of nutsedge. However, its application is limited to peanut (Brecke et al., 1995; Horrall et al., 1995), and the effectiveness of this chemical is influenced by environmental factors and plant age at the time of the application (Horrall et al., 1995). However, residual presence of imazameth in the soil severely reduced cotton yield and significantly retarded the growth of vegetables crops even when the herbicide is applied at 0.072 kg/ha (Strebe et al., 1995). Pereira et al. (1987) provided an excellent review of the various herbicides reported to be used on both yellow and purple nutsedges. They reported that each of the herbicides discussed has practical problems associated with its usage.

Biological control. It is clear that adequate control of weeds, especially problem weeds like purple nutsedge, is becoming increasingly difficult, and the existing control

methods are either not very effective in controlling this weed or are very costly. Since purple nutsedge is difficult to control and has the ability to produce a monotypic stands, it is an excellent candidate for biological control. Biological control using natural enemies is not a new concept. Unfortunately, in the past few decades, it has not been seriously sought because of the perceived effectiveness of chemical herbicides and farmers' expectation of weed-free fields. However, due to over-reliance on these herbicides, which control only some annual and perennial weeds, new populations of weeds emerge that are difficult to control with presently available methods. There are several compelling reasons to seek a biological control for purple and yellow nutsedges. These include, limitations in the effectiveness of available chemical herbicides to control these weeds, the cancellation of the use of methyl bromide as a general purpose soil fumigant, phasing out of several older herbicides during re-registration, the high cost of developing and registering new chemical herbicides, the emergence of herbicide-resistant weeds, and the increasing demand from the concerned public for environmentally friendly alternatives to chemical pesticides.

Biological control of weeds utilizes living organism(s) to attack a weed population and maintain it at or below the desirable levels. Two major strategies are employed: the classical strategy is an ecological approach which relies on the ability of an organism to multiply and spread following small-scale releases. The disease then reaches epidemic proportions and gradually attains a state of balance with the target weed, keeping the latter at an acceptably low level (Watson, 1994; Charudattan, 1990; 1991). The bioherbicide strategy, a major strategy of biological control of weeds in recent years,

aims to attack the weed population with a single or multiple nonpersistent applications of inoculum (Charudattan, 1990; 1991). The inoculum inundates a large weed-infested area to increase the effectiveness of the agent. The inoculum is formulated to reduce the effects of desiccation and to improve performance, and it is applied, usually annually, similar to the manner of application of a chemical herbicide. Generally, the pathogens considered for this strategy are fungi; hence the commonly used name mycoherbicides.

The concept of using inundative inoculation with a pathogen for weed control has gained momentum in recent years. Research in the late 1960s and through the 1980s culminated in the successful registration of two plant pathogens as bioherbicides and their subsequent use in commercial agriculture for nearly two decades (Charudattan, 1991). Between 1980 and 1997, five bioherbicides were registered in the United States, Canada, and Japan: DeVine® (*Phytophthora palmivora* [Butler] Butler to control *Morrenia odorata* Hook. & Arn.) Lindl. in citrus in Florida), Collego® (*Colletotrichum gloeosporioides* [Penz.] Sacc. f. sp. *aechynomene* for *Aschynomeme virginica* (L.) B.S.P. in rice and soybean in Arkansas), BioMal® (*C. gloeosporioides* f. sp. *malvae* for *Malva pusilla* Medik. in various crops in Canada), Dr. BioSedge® (*Puccinia canaliculata* [Schw.]. Lagerh.) for *Cyperus esculentus* in various crops in the United States), and the newest product, Camperico®, a bacterium (*Xanthomonas campestris* Migula pv. *poae*) to control annual bluegrass (*Poa annua* L.) in turf in Japan (Imaizumi, et al. 1997). Two other fungi have been approved by local authorities to control weedy tree species: Biochon® [*Chondrostereum purpureum* (Pers.:Fr.) Pouzar] to control *Prunus serotina* Ehrh. in Dutch forests) and Stumpout® [*Cylindrobasidium laeve* (Pers.:Fr.) Chamuris to

control unwanted broadleaf tree species in tree plantations in South Africa]. Both bioherbicide agents are used as cut-stump treatments to prevent resprouting.

There is extensive literature on the use of the bioherbicide tactics to control weeds (Charudattan, 1991). However, there are only a few research papers on the use of bioherbicide agents to control purple nutsedge. The earlier works on biological control of purple nutsedge dealt with the use of two insect pests of nutsedge, *Bactra truculenta* (Meyrick) (Lepidoptera: Torticidae) and *Athesapeuta cyperi* Marshall (Coleoptera: Curculionidae). However, this effort was later abandoned because the insects were ineffective in controlling nutsedge (Goeden, 1978). Doll (1983), Goeden (1978), Frick and Chandler (1978), and Frick et al. (1978) did extensive studies on the moth larvae *Bactra verutana* Zeller (Lepidoptera: Torticidae), a predominant natural enemy of purple nutsedge. These efforts were also abandoned due to low larval density, lack of sufficient damage, high nutsedge survival despite larval infestation (Frick, 1978), and infestations occurring only late in the season (Frick and Garcia, 1975; Frick and Chandler, 1978; Frick et al., 1978). Another attempt to utilize this insect by coating the larvae with the herbicide glyphosate gave inconclusive results (Quimby and Fick, 1980).

Development of *Puccinia canaliculata*, as bioherbicide for yellow nutsedge, was started in 1979 by Phatak and his coworkers in Georgia (Phatak et al., 1981; 1982). This rust was reported to spread very rapidly and could cause 90% mortality of shoots within five weeks (Phatak et al., 1983; 1984). Phatak (1984), Phatak et al. (1984; 1987), Bruckart et al. (1985; 1988), and Callaway et al. (1985; 1987) reported that the rust, when applied in conjunction with glyphosate at low rates, provided good control of certain

biotypes of yellow nutsedge. However, the fungus did not infect purple nutsedge (Scheepens and Hoogerbrugge, 1991) or several yellow nutsedge biotypes that are naturally resistant to certain physiologically specialized strains of this rust (Bruckart et al., 1988).

A number of other plant pathogens are reported to occur on nutsedge (Evans, 1987; 1995; Barreto and Evans 1995a; 1995b; Phatak, 1987). Some have been documented as potential bioherbicides to control purple nutsedge based on field observations, but no further attempts to develop these pathogens have been undertaken (Evans, 1987; Barreto and Evans, 1995a; 1995b; Phatak, 1987). Pathogens such as *Bipolaris spicifera* (Bain.) Subra., *Cercospora caricis* Ould., and *Curvularia lunata* (Wakker) Boedijn have been studied (Blaney and Van Dyke, 1987; Shelby and Bewick 1991), but their potential as bioherbicides remains inconclusive.

The overall objectives of this dissertation were to 1) screen fungi that were pathogenic to purple nutsedge and choose a potential bioherbicide candidate; 2) determine the epidemiological factors that affected the efficacy of this pathogen; 3) determine the ability of the pathogen to reduce purple nutsedge interference with crop; and 4) determine the field efficacy of this bioherbicidal agent.

CHAPTER 2
SCREENING FOR FUNGAL PATHOGENS OF NUTSEDGES

Introduction

There are very few reports of the occurrence of pathogens on purple nutsedge (Barreto and Evans, 1995a; 1995b; Evans, 1987; Phatak et al., 1987). Although these pathogens were suggested as potential biological control agents for purple nutsedge, this assessment was based merely on field observations; the biological control potential had not been tested. Active research on the use of fungal plant pathogens to control this weed has been undertaken only recently in the United States, Brazil, and Israel. Shelby and Bewick (1991), working on *Curvularia lunata* (Wakker) Boedijn, reported this organism to be pathogenic to purple nutsedge. However, further development of this pathogen as a bioherbicide was terminated due to lack of efficacy. Upadhyay et al. (1991) reported the potential of *Ascochyta cypericola* sp. nov. which caused leaf blight on purple nutsedge, but they did not pursue this agent further. Blaney and Van Dyke (1987) isolated *Bipolaris specifera* (Bain) Subram., *Cercospora caricis* Ould., and *C. lunata* from yellow nutsedge (*C. esculentus* L.) in North Carolina, and found that only *C. caricis* was a credible biocontrol agent of this weed. Phatak et al. (1983; 1987) and Beste et al. (1992) reported yellow nutsedge, which is also a major weed in the temperate and tropical regions of the world, can be successfully controlled in some instances by *Puccinia canaliculata* (Schw.) Lagerh. Unfortunately this rust fungus was effective only to certain

biotypes of yellow nutsedge and was not effective at all on purple nutsedge. Stoval and Clay (1987) reported the infection of the inflorescence of purple nutsedge by *Balansia cyperi* Edg.. However, the biocontrol potential of this fungus appeared to be limited. The infected plants had numerous small tubers compared to noninfected plants. Moreover, the effect of this pathogen on shoots and tuber weight was very negligible. The infected plants were more vigorous and represented a more serious weed problem than noninfected nutsedge. The increased growth can result in a greater competitive ability with respect to crop plants, and increased tuber production would result in a larger population of tubers in the soil. A leaf rust caused by *Puccinia romagnoliana* Marie and Sacc. has been reported as a potential biological control candidate (Bedi and Sokhi, 1994) for purple nutsedge. Under greenhouse conditions, this rust significantly reduced the numbers and weight of tubers. However, like other obligate pathogens, the problems with inoculum production on a solid substrate has halted the development of this pathogen as a potential bioherbicide.

Research on the biocontrol of purple nutsedge through inundative application of indigenous fungal pathogens has been in progress for less than two decades. Presently, no bioherbicides are being used to control purple nutsedge, although Dr. Biosedge (*Puccinia canaliculata*) has been registered for this purpose. There have been no reports of phytotoxin production by a nutsedge pathogen or the possible utility of phytotoxin to control purple nutsedge. Against this background, in 1993, a biological control weed program was initiated by Dr. Charudattan under the sponsorship of a CBAG grant (see Acknowledgments), to evaluate the possibility of utilizing indigenous fungal pathogens

to control purple nutsedge. The research reported here is an outcome of this program. In this chapter of the dissertation are presented the results of the screening for the best fungal candidate to be used for further development as a bioherbicide based on the pathogenicity and host range.

Materials and Methods

Isolation and Screening

Diseased leaves of nutsedge plants were collected from various locations in Florida in June, July, August, and September 1994 and kept in a cold room for 2 days. The leaf pieces (4 mm^2) were cut, surface sterilized with 0.5% sodium hypochlorite solution, rinsed twice with sterile water, and plated either on 1% water agar or potato dextrose agar (PDA; Difco, Detroit, MI) or incubated on wet filter paper. After three days of incubation, fungi that grew from the lesions were isolated and transferred to fresh PDA. Pure cultures of the recovered fungi were prepared from hyphal tips or single conidium and maintained on half-strength PDA slants and in soil in test tubes as stock cultures. The isolated fungi were identified to the genus based on their conidial morphology and growth characteristics on media.

Nutsedge Plant Production

Pathogenicity of the isolated fungi was tested on both yellow and purple nutsedge plants. Purple nutsedge plants used in this experiment were grown from tubers collected from Florida (Gainesville), Puerto Rico, and the Virgin Islands, and yellow nutsedge

plants from tubers obtained from the collection of Dr. Thomas Bewick (Dept. of Horticultural Science, University of Florida, Gainesville, FL). To ensure uniform plant size, tubers of uniform size and weight were used. The weight ranged 0.5-0.75 g for tubers of purple nutsedge and 0.3-0.5 g for tubers of yellow nutsedge. The plants were grown to uniform growth stages by sprouting the tubers before transplanting. The tubers were sprouted as follows: they were submerged in water in shallow trays for 1 week, then the water was drained, and the trays were covered with plastic wrap to maintain moisture and high humidity. Tubers were rinsed every other day until they sprouted. When enough tubers were sprouted to plant a treatment, the shoots were trimmed to 1 cm in length. Five tubers were planted in a 10-cm-diameter pot containing a commercial potting medium (Metro-Mix 300; W.A. Grace & Co, Cambridge, MA). The pots were kept on a greenhouse bench under proper conditions of water and nutrients.

Inoculum Production

For inoculum production, a small piece of agar plug with mycelium was taken from the stock culture of each isolate and aseptically transferred to a fresh PDA plate. The plate was sealed with Parafilm (American National Can, Greenwich, CT) and incubated in the dark at 28°C for 7 days. Agar plugs (6-mm diameter) from the margins of young colonies were used as seed inoculum. Potato dextrose agar (PDA) and V-8 juice agar (200 ml V-8 Juice, 18 g agar, 800 ml water, and 3 g $CaCO_3$) were used for conidial production. Agar plugs from a 7-day-old culture were placed in the center of each petri plate, the plates then were sealed with Parafilm, and incubated at 28°C in a 12 h

dark/12 h light cycle. Conidia were harvested 15 days after incubation. The plates were flooded with 10 ml of distilled water and the surface of the colonies scrapped with a rubber policeman. The resulting suspensions were filtered through a layer of cheesecloth and the final conidial concentrations were adjusted to 10^5 conidia/ml or 10^6 conidia/ml with a hemacytometer. Sprayable inoculum in the form of a mycelial suspension was used for *Cercospora* spp., which did not sporulate readily. The mycelial suspension was produced by growing the fungus on potato dextrose broth (PDB) and V-8 juice broth in Roux bottles for 15 days at room temperature with no supplemental light. The mycelium was then harvested, the liquid drained, and the mycelium rinsed with deionized water while it was filtered through cheesecloth. The mycelium was gently pressed to remove the excess fluid and 5 g of the mycelium were blended in 1 liter of water in a Waring food blender for 30 seconds at medium speed.

Pathogenicity Testing

Young, actively growing yellow nutsedge and purple nutsedge plants were kept in the greenhouse at 35/25 ± 5°C day/night. Plants at the 4- to 6-leaf-stage were inoculated with a spray of 10^5 to 10^6 conidia/ml, except for *Cercospora* spp. (Table 2-1), in 0.02% (v/v) Silwet L-77® (polyalkyleneoxide modified heptamethytrisiloxane. Silwet is in the family of silicone-polyether copolymer, obtained from Loveland Industries, Greeley, CO), a nonionic organosilicone surfactant used as a carrier. The plants were inoculated to run-off either using a paint brush or an aerosol sprayer (SPRA-TOOL® 8112; Crown North American Professional Products, Chicago, IL). Control plants were sprayed with

water plus 0.02% (v/v) Silwet L-77. The plants were held in a dew chamber at 100% relative humidity at 25 ± 5°C in the dark for 24 h. Subsequently, the plants were removed and placed in a greenhouse having a temperature of 35/25 ± 5°C day/night and, 85 ± 5% relative humidity. The pots were arranged in a completely randomized design with three replications per treatment. Susceptibility of the plants was assessed daily at 4 to 15 days after inoculation based on the development of disease symptoms.

Identification and Characterization

Further identification and characterization were limited to the isolates that were confirmed to be highly pathogenic to purple nutsedge by applying Koch's postulates. The most highly pathogenic isolate was determined to be *Dactylaria higginsii* (Luttrell) M.B. Ellis. The morphologies of the conidia and conidiophores were determined for *D. higginsii* grown on PDA, from plants infected in the greenhouse or the field. The conidia and conidiophores from PDA-grown isolate and from plants infected in the greenhouse were obtained by placing a 0.25-cm^2 plug from an agar slant in the center of each PDA plate. The culture was allowed to grow for 10 days at 28°C with a 12 h light/12 h dark period. The conidia from five inoculated plates were collected for characterization purposes and the rest of the plates were used to inoculate purple nutsedge. The fungus was reisolated from infected plants. Leaves from plants naturally infected in the field were also included to determine the variability in conidia and conidiophore shapes and sizes. Infected leaves were sterilized in 5% sodium hyphochlorite and incubated on moist filter paper in petri dishes for three days. The conidia and conidiophores were scraped

from these leaves with a scalpel and transferred onto glass slides to view under the microscope. The conidia and conidiophores were also washed from PDA and leaf surfaces with sterilized distilled water and the resulting suspension was filtered through two layers of cheesecloth. The suspension was then mixed thoroughly before a drop of the suspension was placed on a glass slide. The measurements were taken by using a calibrated ocular micrometer and counting 100 conidia and conidiophores from each plate or infected leaf. The morphology and dimensions of the conidia and conidiophores were then used for comparison with other *Dactylaria* spp. and *Pyricularia* spp. as described in literature.

Host-Range Determination

The screening for host range and host specificity is the most crucial step in any biological control program. Because of the overriding importance of safety, the greatest care has to be taken to select appropriate test plants and to design screening tests. The host-range determinations were performed only on isolates confirmed in the test of Koch's postulates. The host-range was determined based on the scheme developed by Wapshere (1974). The aim was to select plant species that were potential hosts of the organism in question.

The host-range test list included plants related to the target weed and other recorded hosts of the candidate agent, distantly related species and crop plants that were commonly associated with the target weed. In this case, *D. higginsii* was first tested against susceptible sedges, purple nutsedge being used as a control plant. Susceptibility

to this fungus was also tested on the following plants: Cypereceae: *Cyperus esculentus* L. (yellow nutsedge), *C. globulosus* Aublet. (globesedge), *C. compressus* L. (annual sedge), *C. iria* L. (rice flatsedge), *C. surinamensis* Rottb., *C. papyrus* L., *C. brevifolius* (Rottb.) Hassk *(= Kyllinga brevifolia* L., green kyllinga), *Carex fissa* Mack. var. *aristata* Herman., *Carex* spp., and *Psilocarya nitens* (Vahl.) Wood. Crop plants in which nutsedges are commonly a problem were also included in this test. These plants were: Cruciferae: *Brassica oleracea* L. var. *capitata* (cabbage); Curcubitaceae: *Curcubita pepo* L. (squash); Fabaceae: *Glycine max* (L.) Merr. (soybean), *Pisum sativum* L. (pea); Musaceae: *Musa* sp. (banana); Solanaceae: *Capsicum annuum* L. (bell pepper), *Lycopersicon esculentum* Mill. (tomato), *Solanum tuberosum* L. (potato); Poaceae: *Avena sativa* L. (oat), *Hordeum vulgare* L. (barley), *Oryza sativa* L. (rice), *Saccharum officinarum* L. (sugarcane), *Sorghum vulgare* Pers. (sorghum), *Triticum aestivum* L. (wheat), and *Zea mays* L. (corn). Since *D. higginsii* was previously placed in the genus *Pyricularia*, which is pathogenic to grasses, other members of the Poaceae reported as hosts of *Pyricularia grisea* were also included in this test. These plants include the following: *Cenchrus echinatus* L., *Digitaria bicornis* L., *D. ciliaris (*Retz.) Koel., *Echinochloa crusgalli* (L.) Beauv., *Eleusine indica* (L.) Gaertn., *Lolium perenne* L., *Panicum maximum* L., *Paspalum notatum* Flugge., *Setaria faberi* Herm., *Setaria italica* (L.) Beauv., *Sorghum halepsense* (L.) Pers., and *Zizania aquatica* L. Five plants of each species or variety were inoculated with suspensions of 1 x 10^6 conidia per ml in 0.02% (v/v) Silwet L-77. Control plants were sprayed with water plus 0.02% (v/v) Silwet L-77. Inoculum was applied to the foliage of test plants to runoff with a 32-oz plastic spray bottle. Inoculated plants were

incubated in a dew chamber for 24 h before they were transferred to a greenhouse bench. The pots were arranged in a completely randomized design with four replications per treatment. The age of the inoculated plants varied from 2 to 4 weeks depending on the growth habit of the species and plant size suitable for inoculation.

Disease Assessment

Host reaction to *D. higginsii* was determined from the level of disease development on the inoculated plants. Disease assessment was based on the number of plants affected among the total plants inoculated (disease incidence, expressed as the percentage of diseased plants (Horsefall and Cowling, 1987; James, 1974; Kranz, 1988)), plant reactions to disease based on reaction types, and disease severity (area of plant tissue that is diseased (Kranz, 1988)). Reaction types denote susceptibility of the plants. Reaction type was rated on a scale of 0 to 4, where 0 = no visible reaction; 1 = minute, pinhead-sized spots; 2 = small brown to dark-brown lesions with no distinguishable centers; 3 = small eyespot-shaped lesions with gray center; and 4 = typical coalescing lesions, elliptical with gray centers. Type 0 lesion was considered as an immune host reaction; type 1, and 2 as resistant host reaction; type 3 and 4 lesions as a susceptible host reactions. With the type 3 and 4 host reactions, the pathogen was able to produce new conidia on the diseased leaves following incubation.

Disease progress was assessed on all plants in each pot by estimating the proportion of leaves with lesions and necrotic or dying leaves per plant. The latter was expressed as disease severity using a disease rating scale developed by Horsfall and

Barratt (1945), but with a slight modification. In the Horsfall-Barratt scale there are 12 class values which correspond to different levels of disease severity. The rating scale used in the present consisted of 11 class values which represented the percentage of disease severity. The classes used were: 1 = 0; 2= 1-5%; 3 = 5-10%; 4 = 10-15%; 5 = 15-30%; 6 = 30-55%; 7 = 55-65%; 8 = 65-75%; 9 = 75-85%; 10 = 85-95% and 11 = 95-100%. The mean class value was used to determine the final disease severity value. The final severity values were obtained from the calibrated curves with grade numbers on the X-axis and percentage disease on a semi-log. Y-axis with one and one-half phases from either end up to 50 percent. Disease severity was assessed every 2 days after inoculation until disease development remained constant, i.e., 30 days after inoculation.

Effect of *D. higginsii* on the Growth of Purple Nutsedge

The effect of spraying *D. higginsii* on the growth of purple nutsedge was assessed by clipping the living above-ground shoots at the soil level and washing the tubers to clear the soil clinging to the roots and tubers. The roots were trimmed leaving just the bulbs and tubers for drying, 45 days after inoculation. The shoots and the tubers from individual pots were placed in paper bags and oven-dried at 70°C for a week. Dead plant tissue was not included in the dry-weight measurement. The dry-weight data were expressed as the percentage reduction in biomass compared with the biomass of the uninoculated control.

Data Analysis

All experiments were performed twice unless stated otherwise. A randomized complete block design with four replications was used for all experiments. All percentages were arcsine-transformed prior to analysis (Gomez and Gomez, 1984). Since most of the data were quantitative and there was no significant variability among the trials, the data were pooled (homogeneity of variance was tested using the Bartlett test; Gomez and Gomez, 1984). Disease progress values over time were analyzed by transforming with the Gompertz transformation (Berger, 1981; Kranz, 1974a) and the goodness-of-fit of the model was determined from F statistic, R^2 values, and examination of plot residuals. Final shoot numbers, shoot dry weights, tuber numbers, and tuber dry weights were used to determine the effect of *D. higginsii* on the growth components of purple nutsedge. Initial analysis of variance of various weed-growth components did not show any variability between repeated trials. Therefore, the data from these trials were pooled and the treatment means showing significant effects were separated by Fisher's protected least significant difference test at the 5% level of significance.

Results

Isolation, Pathogenicity, and Characterization

Only a few genera of fungi were consistently isolated from the diseased nutsedge plants collected from around Florida (Table 2-1). *Cercospora caricis*, was obtained from Dr. Greg Weidemann, University of Arkansas, Fayetteville, Arkansas, and *Cercospora* sp. was obtained from Dr. Doug Boyette, USDA-ARS Southern Weed Science

Laboratory, Stoneville, Mississippi. The pathogenicity of these fungi was tested on yellow and purple nutsedges, but none of the fungal isolates was capable of causing disease on any of the inoculated plants. Thus, Koch's postulates could not be fulfilled, indicating that none, including the two isolates of *Cercospora* sp. which were previously reported to be pathogenic to yellow nutsedge (Boewe, 1964; Blaney and Van Dyke, 1987; Chupp, 1953; Greene, 1953; Thirumalachar and Govindu, 1953), was pathogenic to any of the yellow or purple nutsedge test plants.

An exception was an isolate of a fungus found in late September, 1994, from diseased purple nutsedge leaves collected in Gainesville. It was highly pathogenic to both yellow and purple nutsedge plants spray-inoculated with a suspension containing conidia and mycelia. The symptom first appeared on the inoculated plants 4 to 5 days after inoculation as tiny, dark-brown flecks on the leaf blades. The lesions enlarged to form elliptical, pale-brown lesions surrounded by a narrow, dark-brown border. The lesions measured 1.5-7 x 0.5-2 mm. The lesions coalesced to form larger irregular lesions and blotches and the leaves died back from the tips (Fig. 2-1). Most of the leaves were dead 15-20 days after inoculation. Under greenhouse conditions, a gray, velvety layer of conidiophores and conidia developed on both surfaces of the lesions and secondary infections occurred 10 days after the appearance of the initial symptoms. These symptoms observed under greenhouse conditions were similar to those seen in the field. The mycelium grew internally in the infected tissue. The conidiophores emerged through the epidermal cells, either singly or in clusters. The conidiophores were inflated at the base and tapered toward the apex (Fig. 2-2); they were 0-1 septate and were

hyaline. At the apex, a crown of 2-4 dactyliform denticles were formed on which the conidia were borne. The conidiophores produced 2-3 successive whorls of conidia (Fig. 2-2). The average dimensions of the conidia and conidiophores from the field samples were 28.66 x 6.6 *um* (width at the widest point) and 45.2 x 7.0 μm (width at the widest), respectively. Conidia and conidiophores from PDA-grown fungus had the average sizes of 29.0 x 6.5μm and 47.0 x 7.0 μm. Diseased leaves obtained from greenhouse had conidia that averaged 28.8 μm x 6.0 μm and conidiophores 46.0 x 7.2 μm (Table 2-2). There was no significant variability among conidia and conidiophores of PDA-grown fungus, fungus from plants that were naturally infected in the field, and fungus from plants inoculated in the greenhouse. The dimension of the conidia and conidiophores of *D. higginsii* were compared with those of *Dactylaria* spp. and *Pyricularia* spp. given in the literature (Table 2-2). There were differences in the dimensions and shape of conidia and conidiophores of *D. higginsii*, but, the dimensions of the conidia and conidiophores fit well within the described range for *Pyricularia. higginsii* (Luttrell, 1954). Hence this fungus was considered to be *Dactylaria higginsii* (Luttrell) M.B. Ellis (= *Pyricularia higginsii* Luttrell).

Host-Range Determination

The host reactions of the plants to *D. higginsii* were determined from the presence of disease symptoms and the susceptibility of the test plants classified on the basis of the reaction types. In repeated experiments, *D. higginsii* was pathogenic to all collections of purple nutsedge (disease incidence of 100% and reaction type 4, very susceptible) and

yellow nutsedge (disease incidence from 80 - 100% and reaction type from 3 - 4). Rice flat-headsedge (*C. iria*), kyllinga (*C. brevifolius* = *Kyllinga brevifolia*), globesedge (*C. globulosus*), and annual sedge (*C. compressus*) were equally susceptible (Table 2-3). These sedges are the most commonly reported weeds of many crops, home gardens, turfgrasses, and fruit trees in the Southeastern United States. *Cyperus papyrus*, which is grown as an ornamental, and *C. surinamensis*, a lesser known sedge, were immune to *D. higginsii*. Since this fungus was initially classified as *Pyricularia higginsii* (Luttrell, 1954), plants in the Poaceae family, which includes several reported hosts to *Pyricularia* spp. were tested to confirm the host range of *D. higginsii*. All of the members of the Poaceae (Table 2-4), which are reportedly hosts to *Pyricularia grisea* Sacc., a species that closely resembles *D. higginsii*, were also immune. Included in this list of immune plants are the important crops like rice, corn, wheat, sorghum, and oat.

Other crop plants tested were also immune to this fungus (Table 2-5). Thus, the results of the host-range study confirmed that *D. higginsii* was nonpathogenic to members of the Poaceae and the different crops species tested. This was indicated by the absence of symptoms on any of the tested plants (infection-type 0).

Disease Progress

The disease progress of dactylaria leaf blight caused by *D. higginsii* on purple nutsedge, as measured by disease severity, is shown in Fig. 2-3A. The fungus had a latent period of 4 days, the disease progress was slow initially, but it increased drastically after 7 days and reached the maximum (85% severity) after the 15 days. After this date,

nearly 100% of the inoculated plants were heavily diseased with 95% severity. Lesions were also visible on the adjacent noninoculated control plants, but the disease was confined to a few older leaves and the severity never reached 5% on these plants. The disease progress was best described by the Gompertz growth model (Fig. 2-3B), and the overall apparent infection rate in the three trials averaged $r_G = 0.1077$ unit per day, (SE = 0.0012, $R^2 = 0.98$; $P<0.001$). Under greenhouse conditions this fungus was capable of causing secondary infections, and the source of the inoculum for secondary infections was from the infected, dying leaves.

Effect of *D. higginsii* on the Growth of Purple Nutsedge

The bioherbicidal potential of *D. higginsii* to control purple nutsedge was validated when purple nutsedge plants that were spray-inoculated with 10^6 conidia/ml developed severe disease and died. The resprouting shoots were later reinfected and killed. The effect of inoculation with *D. higginsii* was apparent from the reduction in the shoot and tuber production of purple nutsedge compared with the noninoculated control. *Dactylaria higginsii* had caused significant reductions in shoot and tuber numbers by 73% and 80% (Figure 2-4), and shoot and tuber dry weights by as much as 71% and 67%. Since *D. higginsii* has the capability to reduce these weed-growth components, *D. higginsii* is considered to have the potential to be a bioherbicide to control purple nutsedge.

Table 2-1. List of fungi isolated from yellow and purple nutsedge plants and their pathogenicity to yellow and purple nutsedges.

Fungal isolate	Host plant	Inoculum concentration tested	Location/Origin	Host reaction	
				Yellow nutsedge	Purple nutsedge
Alternaria sp.	Yellow nutsedge	10^5 conidia/ml	Quincy, FL	-	-
			Gainesville, FL	-	-
Curvularia sp.	Yellow nutsedge	10^5 conidia/ml	Quincy, FL	-	-
			Gainesville, FL	-	-
Fusarium sp.	Yellow nutsedge	10^6 conidia/ml	Gainesville, FL	-	-
Collectotrichum sp.	Yellow nutsedge	10^6 conidia/ml	Gainesville, FL	-	-
Phoma sp.	Yellow nutsedge	10^5 conidia/ml	Gainesville, FL	-	-
Cladosporioum sp.	Yellow nutsedge	10^6 conidia/ml	Gainesville, FL	-	-
Cercospora sp.(873)	Yellow nutsedge	5 g mycelium/l	USDA-ARS, MS	-	-
C. caricis (SDSW)	Yellow nutsedge	5 g mycelium/l	Dr. Weidemann, AR	-	-
Phyllachora sp.	Yellow nutsedge	10^4 spores/ml	Gainesville, FL	-	-
			Leesburg, FL	-	-
Dactylaria higginsii (=*Pyricularia higginsii*)	Purple nutsedge	10^6conidia/ml	Gainesville, FL	+	+

- = Plants did not show any disease symptoms.
+ = Plants developed disease symptoms.

Table 2-2. A comparison of conidia and conidiophore dimensions of *Dactylaria higginsii* with those described for *Pyricularia* spp.

	LENGTH(μm)		WIDTH(μm)	
	Range	Mean	Range	Mean
Conid				
Field sample (*D. higginsii*)	22.2 -33.6	28.6	5.3 - 6.7	6.6
PDA sample (*D. higginsii*)	22.0 - 34.0	29.0	4.4 - 7.7	6.5
Greenhouse sample (*D. higginsii*)	21.9 - 35.6	28.8	5.5 - 7.7	6.0
Pyricularia higginsii[a]	24.3 - 36.5	28.6	5.3 - 6.5	6.0
P. grisea Sacc.[b]	17.0 - 28.0	20.9	6.0 - 9.0	7.6
P. oryzae Cav.[b]	17.0 - 23.0	21.2	8.0 - 11	9.6
P. ebellsii Ellis[c]	28.0 - 35.0	-	13.0 - 15	-
P. grisea (grass) [c]	12.5 - 46.0	-	5.0 - 13.5	-
P. grisea (*Commelina*)[c]	21.5 - 23.0	-	9.0 - 9.5	-
Conidiophores				
Field sample (*D. higginsii*)	37.4 - 55.2	45.2	4.4 - 8.8	7.0
PDA sample (*D. higginsii*)	38.0 - 55.0	47.0	4.4 - 8.8	7.0
Greenhouse sample (*D. higginsii*)	38.0 - 57.0	46.0	4.4 - 9.0	7.2
Pyricularia higginsii[a]	30.4 - 63.8	-	4.6 - 6.1	-
P. grisea[b]	-	150	2.5 - 4.5	-
P. oryzae[b]	-	130	3.0 - 4.0	-

Source:
[a]Luttrell, 1954
[b]Ellis, 1971
[c]Purchio and Muchovej, 1993

Table 2-3. Host-range test of *Dactylaria higginsii* on various members of the Cypereaceae.

Name/Origin	Disease incidence	Reaction type
Cyperus rotundus L.		
Gainesville	100	4
Puerto Rico	100	4
Virgin Islands	100	4
C. esculentus L.		
Gainesville	100	4
Oklahoma	100	4
Texas	100	4
Greenfield, CA	100	4
Frankfurt, KY	85	3
South Carolina	100	4
Maryland	100	4
Hood River, OR	80	3
Ohio	100	4
Pennsylvania	100	4
Franklin, MI	100	4
Mesa, WA	100	4
C. brevefolius Hassk.(= *Kyllinga brevefolia* L.)	100	4
C. compressus L.	100	4
C. iria L.	100	4
C. globulosus Aublet	100	4
C. papyrus L.	100	0
C. surinamensis Rottb.	0	0
Carex fissa Mack. var. *aristata* Herman.	0	0
Carex sp.	0	0
Psilocarya nitens (Vahl.) Wood.	0	0

Table 2-4. Host-range test of various grassy weeds reported as hosts of *Pyricularia grisea*.

Scientific name	Common name	Disease incidence	Reaction type
Brachiaria platyphylla (Griseb.) Nash.	Broadleaf signalgrass	0	0
Brachiaria ramosa (L.) Stapf.	Browntop millet	0	0
Cenchrus echinatus L.	Southern sandbur	0	0
Digitaria ciliaris (Retz.) Koel.	Southern crabgrass	0	0
Digitaria bicornis (Lam.) R. & S.	Tropical crabgrass	0	0
Echinochloa crus-galli (L.) Beauv.	Barnyardgrass	0	0
Eleusine indica (L.) Gaertn.	Goosegrass	0	0
Lolium perenne L.	Perennial ryegrass	0	0
Panicum maximum Jacq.	Guineagrass	0	0
Paspalum notatum Flugge.	Bahiagrass	0	0
Setaria faberii Herm.	Giant foxtail	0	0
Setaria italica (L.) Beauv.	Foxtail millet	0	0
Setaria viridis (L.) Beauv.	Green foxtail	0	0
Sorghum halepsense (L.) Pers.	Johnsongrass	0	0
Zizania aquatica L.	Annual wildrice	0	0

2-5. Host-range test of crop plants in which purple nutsedge is reported as a problem weed.

Scientific name	Common name	Disease incidence	Reaction type
Avena sativa L.	Oats	0	0
Brassica oleracea L. var. Capitata	Cabbage	0	0
Capsicum annuum L. var. Calwonder	Bell pepper	0	0
Curcubita pepo L.	Squash	0	0
Daucus carota L. cv. Nantes.	Carrot	0	0
Glycine max (L.) Merr. cv Hutton	Soybean	0	0
Hordeum vulgare L.	Barley	0	0
Lycopersicon esculentum Mill. cv. Agroset	Tomato	0	0
Musa sp.	Banana	0	0
Oryza sativa L. cv. Calrose Medium Grain	Rice	0	0
Pisum sativum L. cv. Little Marvel	Pea	0	0
Saccharum officinarum L.	Sugarcane	0	0
Solanum tuberosum L.	Potato	0	0
Sorghum vulgare Pers.	Sorghum	0	0
Triticum aestivum L.	Wheat	0	0
Zea mays L. cv. sh2	Sweet corn	0	0

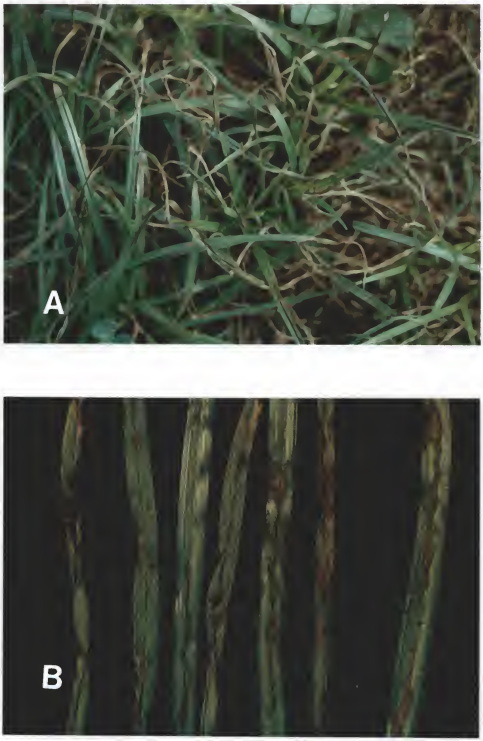

Fig . 2-1. Disease symptoms of Dactylaria leaf blight caused by *Dactylaria higginsii*. Disease symptoms in the field (natural infection) (**A**) and different stages of symptom development (**B**)

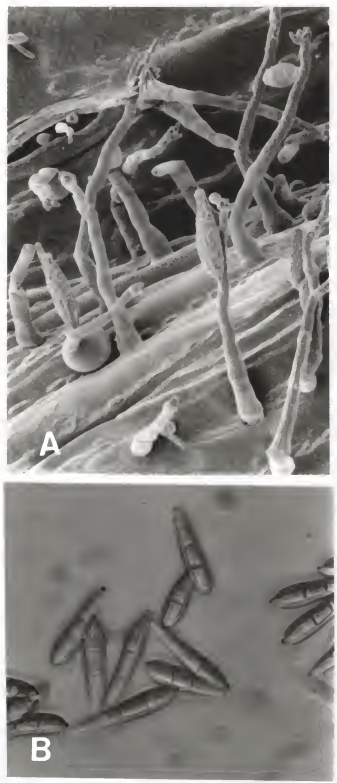

Fig. 2-2. Morphologies of conidia and conidiophore of *Dactylaria higginsii*. Electron micrograph of conidiophores bearing conidia on infected leaf (**A**) and shape and septation of conidia (**B**).

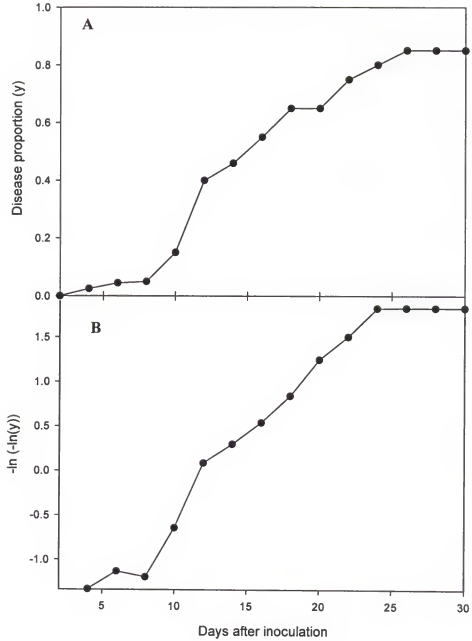

Fig. 2-3. Disease progress curve of dactylaria leaf blight of purple nutsedge caused by *Dactylaria higginsii*. Untransformed severity values (**A**). Severity values transformed using the Gompertz model (-ln (-ln(y))) (**B**). The regression equation for gompit $Y = -1.385 + 0.144X$ ($R^2 = 0.95$).

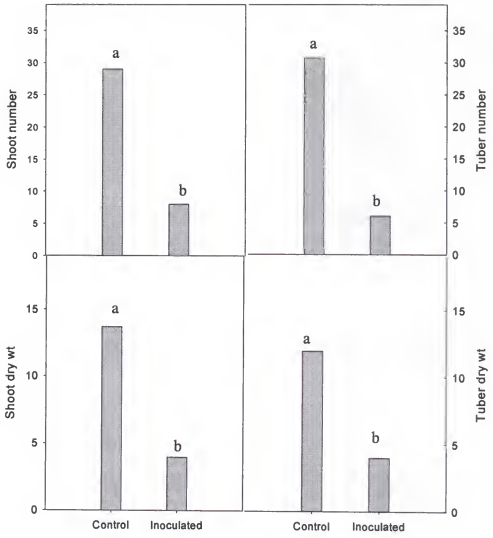

Fig. 2-4. Effect of *Dactylaria higginsii* on weed-growth components. Data from two trials were pooled as the variance was homogenous. Bars represent means of four replicates. Bars within each weed-growth component having the same letters are not significantly different according to Fisher's protected least significant difference test at $P < 0.05$.

Discussion

Only a few fungal pathogens were isolated from both yellow and purple nutsedge plants and a majority of these were either secondary or weak pathogens. They required the presence of wound or other avenues for infection, or they were saprophytes in the necrotic tissues on the leaves. Only one isolate had promising bioherbicidal activity towards yellow and purple nutsedges and it was identified as *Dactylaria higginsii* (Luttrell) M.B. Ellis. In repeated trials, *D. higginsii* was confirmed, by applying to Koch's postulates, to be the casual agent of a blight-like disease of purple nutsedge observed in the field. This fungus sporulated prolifically on both PDA and V-8 juice agar, but growth on V-8 agar was not very extensive.

Tubers and bulbs have very important roles in propagation and dissemination of purple nutsedge. Moreover, the presence of the shoots and the tubers affect crop yields through production of allelopathic substances and by competition for moisture, nutrients, and space. Competitiveness of this weed is influenced by both the shoots and the tubers, and a control method that can simultaneously reduce the numbers of both the shoots and the tubers will reduce the competitiveness of this weed. *Dactylaria higginsii* was very effective in not only infecting and killing the shoots, but also in reducing or keeping the tuber number and tuber weight at low levels. This translates to a reduction in the potential propagative materials.

The ability of *D. higginsii* to reduce growth components of purple nutsedge was an excellent indicator of the potential of this fungus as a bioherbicide to control this

weed. In the greenhouse, purple nutsedge plants were never able to resume normal growth after 20 days following inoculation. Inoculated plants did not grow to maturity, probably due to reduction in accumulated sugars and starch. This was indicated by significant reduction in the size, dry weight, and numbers of tubers when compared to the noninoculated control treatment. The results obtained from this experiment corroborated the findings of Santos et al. (1997a) who reported that smaller tubers (<0.75 g) did not resprout (total depletion) after removing the shoots several times.

The suppression of shoot growth prevented the development of shoots into mature plants and translocation of starch for tuberization. Thus, the effectiveness of *D. higginsii* as a bioherbicidal agent may be related to its indirect effect on tuber formation and storage of energy reserves. It is known that purple nutsedge plants exhaust considerable amounts of stored energy during sprouting and shoot growth. Any condition that forces nutsedge plants to produce new growth, for example to compensate for the photosynthetic tissue lost due to infection by *D. higgginsii*, will cause a depletion in the stored energy reserves. According to William and Bendixen (1987), 60% of the sugar and carbohydrates reserved in the tubers were consumed during the first sprouting, while about 20% were used in the subsequent sprouting. Carbohydrate reserved in the tubers are constantly used up by the sprouting shoot, causing the tubers to become small and less vigorous and predisposing some of the tubers to infection by secondary pathogens. Porter (1995) found several soil-borne pathogens that had the potential to attack the stressed tubers.

The ability of *D. higginsii* to induce severe leaf infection within four days after inoculation further indicated that this pathogen has the potential to be developed as a bioherbicide. The symptoms consisted of small watery spots on the foliage which later coalesced to cause necrotic leaf bight. The infected tissues were subsequently killed. Bailey and van Eijnatten (1961) and Purchio and Muchovej (1993), observed similar types of disease symptoms on corn and on *Commelina agraria* L. infected with *Pyricularia grisea*. However, *Dactylaria higginsii* differs from *P. grisea*, considered by Luttrell (1954) as the type species of the genus *Pyricularia*, in that the conidia of the former fungus occur in whorls of 2, 3, or rarely 1, at the apex of the conidiophores. The conidia of *P. grisea* are often so closely grouped that they appear to be produced in heads (Ellis, 1971). The conidia of *D. higginsii* also are abstricted from short strigmata. In *P. grisea* the conidia are likewise borne on short stipes, but these stipes resemble disjunctor cells. The stipe cell ruptures in the middle, leaving a protuberant collar on the conidiophores and a similar collar at the base of the conidium. The conidia of *D. higginsii* might be considered capitate, a characteristic of the genus *Dactylaria* (Ellis, 1971). According to Luttrell (1954), the obpyriform to obclavate shape of the conidia should be the primary character to separate *Dactylaria* from *Pyricularia*. Nonetheless, he placed this fungus in the genus *Pyricularia* based on the shape of the conidia and because it was a plant pathogen. Ellis (1971) redescribed this fungus as *Dactylaria higginsii* based on the capitate shape of the conidia, its longer, narrower conidia, and the conidial length/width ratio of more than 4. The maximum diameter of the conidia of *D. higginsii* is less than 7.0 μm, which is much less compared to that of *P. grisea* and other closely

related *Pyricularia* spp. (Table 2), which had minimum conidial diameter of 7.0 μm (Ellis, 1971). The conidia length /width ratio for *D. higginsii* is 4.4 (calculated from Table 2) which fits in the range of the conidia length/width described for *P. higginsii*.

De Hoog and Van Oorschot (1985) reassigned *Dactylaria higginsii*, together with three other *Dactylaria* species (*D. junci*, *D. juncicola*, and *D. pyricularioides*) to the genus *Pyricularia* on the basis of rhexolytic conidial secession. However, M.B. Ellis's classification is preferable since the type of conidiation, the morphologies of the conidia and conidiophore, and the growth characteristics of the mycelium on artificial media are unlike those of *Pyricularia* spp. Moreover, *D. higginsii* is not pathogenic to grasses (Poaceae), which is a common features of *Pyricularia* spp. Further study to clarify the classification of the genus *Dactylaria* with particular emphasis on *D. higginsii* at molecular level is therefore recommended.

Host-range is an important step to determine the usefulness of an isolate to be used as a bioherbicide. To be safe, the bioherbicide should not infect crop plant species and other nontarget plants. Therefore, a host-range determination was done based on the protocol developed by Waspshere (1974) to evaluate the potential of *D. higginsii* to be utilized as a bioherbicide to control nutsedge. Unlike the species of *Pyricularia,* which have wide host ranges, the host range of *D. higginsii* is confined only to the genus *Cyperus*. However, some species of *Cyperus* such as *C. papyrus* and *C. surinamensis* were immune. The earlier observation by Luttrell (1954), who reported that this fungus infected purple nutsedge and possibly other related *Cyperus* spp. was confirmed. It did not infect any member of the Poaceae family, such as rice and corn, two major crops

grown throughout the world. Also *D. higginsii* did not infect the other crop plants that were tested. Thus it would be safe to use this fungus as a bioherbicide in many cropping situations. However, further histopathological studies of latent infection on nonhost tissues should be done to confirm the specificity and safety of this fungus if it is to be developed as a bioherbicide. Since *D. higginsii* did not infect members of the Poaceae, this is further justification to keep this fungus in the genus *Dactylaria*, as Ellis (1971) has proposed, rather than in *Pyricularia* as Luttrell (1954) had originally conceived.

The different collections of purple nutsedge did not express any differential susceptibility to *D. higginsii*, unlike yellow nutsedge that was reported to have differential susceptibility to different rust (*Puccinia canaliculata*) strains (Bewick et al. 1991; Bruckart et al. 1988; Phatak et al. 1987; and Scheepens and Hoogerbrugge 1991). The differential susceptibility of yellow nutsedge to the rust fungus has been attributed to the existence of different rust strains and yellow nutsedge biotypes. The finding of nondifferential susceptibility of purple nutsedge to *D. higginsii* may be indicative that the limited number of purple nutsedge collections tested have originated from the same clone. This speculation is supported by the conclusions of Okoli et al. (1997) that the purple nutsedge collections from North America may consist of a clonal population with very negligible variability in the genetic makeup. In the present host-range study, the existence of differential susceptibility among accessions of yellow nutsedge to *D. higginsii* was not obvious. Two out of the twelve accessions of yellow nutsedge (Kentucky and Oregon, Table 2-3) showed 80% and 85% disease incidence with infection types 3 (indicative of their susceptibility), respectively. The differences in disease

incidence of the two yellow nutsedge accessions could be attributed to uneven distribution of inoculum on these plants during spraying or that these plants had passed the susceptible stage which may have allowed some of the plants to escape infection. Shrum (1982) explained that inoculum that is not distributed sufficiently early when the plants are susceptible or the inoculum that is not distributed uniformly have a significant bearing on disease development. The nonuniformity of inoculum distribution on a plant may isolate the pathogen from parts of the plants that are susceptible, thus delaying or preventing disease development. However, further studies of host-pathogen interaction at microscopic level is needed to confirm the pathological reactions of these two accessions of yellow nutsedge to *D. higginsii*.

CHAPTER 3
EPIDEMIOLOGICAL FACTORS AFFECTING THE EFFICACY OF *Dactylaria higginsii* AS A BIOHERBICIDE FOR PURPLE NUTSEDGE

Introduction

Dactylaria higginsii has good potential as a bioherbicide to control purple nutsedge. For this pathogen to be developed as a bioherbicide, it is essential to have a clear understanding of the conditions under which a high level of weed control can be achieved. Several environmental and host-parasite factors can have a direct effect on the efficacy of a pathogen. Collectively, these epidemiological factors need to be determined for the *Dactylaria higginsii*-nutsedge pathosystem. Also, an appropriate inoculum concentration needs to be established, since it has a direct influence on the degree of disease development.

Although disease epidemics had been reported on several pathosystems under natural ecosystem (Kranz, 1974b), these epidemics did not reach catastrophic levels. This phenomenon may be due to the spatial discontinuity of the host and the extremely low level of initial inoculum. The latter results in the discontinuity of inoculum and greater isolation of the host from the pathogen (Shrum, 1982). In effect, the nonuniformity of inoculum distribution isolates the pathogen from a portion of the weed population, thus delaying inoculum buildup.

Environmental factors influence both the activity and persistence of the bioherbicide and probably have the greatest impact on the performances of the

bioherbicide in the field. Most bioherbicides will lose activity rapidly at temperatures above 30°C and will be less active at temperatures below 10°C (Daigle and Connick, 1990). Some agents are effective over even narrower temperature ranges. Development of a formulation that can reduce or overcome the environmental constraint of short, delayed, or absent dew period is an important factor in developing a bioherbicide. However, the formulation must also be compatible with the conventional spray systems.

Dew is important for the germination of the fungal propagules which later cause infections (TeBeest et al., 1978). Free moisture and temperature interact to affect spore germination, infection, disease severity, and subsequent control (TeBeest et al., 1978; Capo, 1982; Capo et al., 1981). Previous reports have indicated that the propagules of phyloplanes microbes declined rapidly on the leaf surfaces due to loss of viability or absence of dew on those leaves (Daigle and Connick, 1990; Andrews, 1992; Kenerley and Andrews, 1990). Since most pathogens require free moisture or high humidity to infect plants, it is expected that the dew period and dew temperature, post-dew incubation period, and incubation temperature will affect disease but at different stages following inoculation. No infection will ensue if dew duration, dew temperature, or incubation temperature is outside the conducive range even though two of the conditions may be near the optima.

A determination of the growth stages at which the host is susceptible to disease development is an important prerequisite for any potential bioherbicidal candidate (Watson and Wymore, 1990). TeBeest et al. (1978), Boyette and Walker (1985), and Charudattan, (1988b) have reported that younger weed seedlings are generally more

susceptible in the host-pathogen systems they studied. However, their findings are contrary to those of Makowski (1993) and Morris (1982) who found that older seedlings are more susceptible to the pathogens in their studies. The more resistant reaction of young seedlings observed by these authors may be due to the seedlings out-growing the disease or to the limited leaf area that could be covered by inoculum. Similar examples of young-plant resistance, compared to more mature plants, are known in other host-pathogen systems (Agrios, 1982; Hafiz, 1952; Kaiser, 1973; Trapero-Casas and Kaiser, 1992)

The objective of this portion of the research was to investigate the optimum conditions for infection, disease development, and satisfactory control of purple nutsedge under the following controlled parameters: 1) inoculum concentration, 2) type of formulation/amendment, 3) dew-period temperature and weed growth-stage and, 4) dew-period duration and weed growth-stage.

Materials and Methods

Inoculum Production

Inoculum of *D. higginsii* was produced on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) amended with 3.7 mg/ml streptomycin sulfate and 2.5 mg/ml chloramphenicol. Conidia were collected from a 14-day-old culture by scraping the agar surface with a rubber policeman. The conidial suspension was then passed through a single layer of cheese cloth. A suspension containing 10^5 conidia/ml was then sprayed using air propellant onto food trays (34 cm x 42 cm), each containing about 300 ml of

PDA. The inoculated trays were then covered with sterilized plastic sheets and incubated at 28°C for 14 days under a 12-h light/12-h dark cycle. The conidia were collected from the trays by flooding the agar surface with 150 ml of sterilized water and scraping with a rubber policeman. The suspension was then passed through a layer of cheesecloth and the final spore concentration was adjusted using a hemacytometer.

Plant Production

Purple nutsedge plants were produced by sprouting the tubers before transplanting. Tuber sprouting was accomplished by submersing the tubers in water in shallow trays for 1 week. The water was then drained, and the trays were covered with plastic sheets to maintain moisture and a high level of humidity. Tubers were rinsed every other day until they sprouted. When enough tubers were sprouted to plant a treatment, the shoots were trimmed to 1 cm in length. Five tubers were planted in a 30-cm-diameter pot containing a commercial potting medium (Metro-Mix 300; W.A. Grace & Co, Cambridge, MA). This technique produced uniform and rapid germination of purple nutsedge plants. The pots were kept on the greenhouse bench with nonlimiting conditions of water and nutrient.

Effect of Conidial Concentration on Disease Development

Purple nutsedge plants at the four-leaf stage were inoculated with six conidial concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 conidia/ml suspended in 0.5% Metamucil (Procter & Gamble, Cincinnati, OH), a humectant, as the standard carrier. A control

consisting of no conidium, but containing 0.5% Metamucil, was included. The plants were inoculated by spraying the conidial suspension with a hand-operated pump sprayer until incipient runoff. Control plants were sprayed similarly with a suspension containing the humectant only without the fungus. After inoculation, the plants were placed in a dark dew chamber at 28°C with 100% moisture for 24 h and then returned to the greenhouse (conditions mentioned earlier). Disease development was monitored for 30 days after inoculation, and the disease was rated daily. The effect of different inoculum concentrations on the growth component of purple nutsedge was determined 45 days after inoculation. The shoot were cut at the soil line and all the tubers and bulbs were collected (counted as tubers). The plant materials were oven-dried at 70°C for 7 days and the dry weights of the shoot and the tubers were recorded.

Effect of Different Amendments on Spore Germination and Disease Development

The type of formulation can influence the efficacy of the biocontrol agents. In this experiment, six types of formulations and three combinations of the formulations were studied for their effects on spore germination and disease development. Appropriate controls were included. The formulations included two surfactants: Silwet® L- 77 (0.02% v/v) (OSI Corporation, Loveland Industries, Inc., Greeley CO), which is a polyalkyleneoxide modified heptamethyltrisiloxane, in the chemical family of silicone-polyether copolymer and Triton® X-100 (0.02% v/v) (Sigma Chemical Company, St Louis, MO) which is t-occylphenoxypolyethoxyethanol in the chemical family of polyoxyethylene ethers. Metamucil® (0.5% w/v) is a plant-derived polysaccharide in the

chemical family of hydrophilic mucilloid, available in most drugstores and supermarkets in the United States. N-Gel® (0.5% w/v) (Hercules Inc., Wilmington, DE) is a nonionic derivative of cellulose. Kelzan® S (0.5% w/v) (Division of Nutrasweet, Kelco Co., San Diego, CA), is a xanthan gum, derived from the exocellular mucilaginous material produced by common bacteria belonging to the genus *Xanthomonas*. Natrosol® (0.5% w/v) (Aqualon Co., Wilmington. DE) is a nonionic hydroxyethylcellulose. Silwet L-77 and Triton X-100 are nonionic surfactants and Metamucil, N-Gel, Kelzan S, and Natrosol are humectants. These substances were added at the rates mentioned above to a suspension of 10^6 conidia. The formulations were then sprayed on the plants until runoff.

The viability of the spores in these suspensions was studied by spraying the conidial suspension on 1.5% water agar in a petri dish. The dishes were then incubated for 24 h in the dark at a constant temperature of 28°C. The percentage of conidial germination in each dish was determined by observing 100 conidia per dish in random fields of a microscope (x 200). No additional germination was observed after 24 h. A conidium with germ-tube of more than half the length of the conidia was considered germinated (Arauz and Sutton, 1989). If more than 100 conidia were present in a field, all conidia were scored to ensure against bias.

Effect of Dew-Period Temperature and Weed-Growth Stage

Three plant-growth stages were included in this study: 4-leaf stage, 6-leaf stage and 8-leaf stage. To achieve plants with these growth stages, the sprouted tubers were trimmed and were planted at four days interval in pots filled with commercial potting

soil. The plants were inoculated with a suspension of 10^6 conidia/ml with 0.5% (w/v) Metamucil added as a humectant. Control plants were inoculated with a suspension of 0.5% Metamucil only. The plants were then given a 24-dew (100% relative humidity) period in an unlighted dew chamber at 15, 20, 25, 30, and 35°C. Upon removal from the dew chamber the plants were kept in a greenhouse at 28 ± 3°C. Disease development was rated as disease severity by methods explained earlier (Chapter 2). The number of days for the inoculated plants to reach 50% disease severity was determined from the disease progress curve.

Effect of Dew-Period Duration and Weed-Growth Stage

Plants at the 4-, 6-, and 8-leaf growth stages were included in this study. The plants were sprayed until run off with a suspension of 10^6 conidia/ml containing 0.5% (w/v) of Metamucil as a humectant. Appropriate controls were included. The plants were incubated at 28°C dew-temperature for 6, 12, 18, and 24 h of dew-period durations. One treatment was not placed in the dew chamber and served as the 0-h dew-period treatment. The dew chamber was kept at 100% relative humidity. The plants were moved to a greenhouse bench at 28 ± 3°C and disease development was rated from 4 to 22 days after inoculation. The numbers of days to reach 50% disease severity was determined as mentioned in the paragraph above (page 56).

Data Analysis

All experiments were done twice and the treatments in each experiment were replicated four times with five plants per replicate. The experiment was carried out in a completely randomized block design. The data were analyzed with the Statistical Analysis System (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed for each experiment. The percentage data were arcsine-transformed and the data on spore concentrations were log-transformed before analysis. Regression analysis of means, orthogonal polynomial contrasts (linear, quadratic, and cubic), and protected Fisher's least significant difference test, $P= 0.05$, were performed when appropriate.

Results

Effect of Conidial Concentration

The effect of conidial concentration on disease development and severity was determined by spraying ten-fold concentrations of conidia starting with 0 as the control and 10^7 as the maximum concentration on the 4- to 6-leaf stages of purple nutsedge plants. Disease was assessed as disease severity daily until 30 days after inoculation. The average disease severity increased with 10-fold increases in conidial concentration up to 10^6 conidia/ ml (Fig. 3-1). The average disease severity decreased at 10^7 conidia/ ml. The relationship of disease severity and conidial concentration could be best described by the second-order polynomial (Fig. 3-1). The residual error from regressions was sufficiently small to discount for higher polynomial factors.

The area under the disease progress curves (AUDPC) also increased with increasing conidial concentration and decreased when the concentration reached 10^7 conidia/ml. No disease developed in the control plants and in plants inoculated with 10^2 conidia/ml as indicated by the zero AUDPC value (Table 3-1). The highest AUDPC value was observed in plants inoculated with 10^6 conidia/ ml. The lowest infective inoculum level was 10^4 conidia/ ml (Table 3-1). A similar pattern was observed for the apparent infection rate, where the fastest progress rate was obtained from plants inoculated with 10^6 conidia/ ml (Table 3-1).

Regression analyses of the effect of conidial concentration on weed-growth components were illustrated in Table 3-2 and Fig 3-2. The growth components of purple nutsedge were significantly affected by the conidial concentration ($P = 0.0001$ and $R^2 = 0.88$, Table 3-2). The regression coefficient for conidial concentration is significantly different from zero ($P = 0.0001$), which indicated that the decrease in weed-growth component was due to the effect of conidial concentration. Weed-growth components were reduced as the conidial concentration is increased. The effect of conidial concentration was intense on tuber dry weight (slope = -2.78) compared to shoot dry weight (slope = -0.85, Fig 3-2). There were no observable differences in shoot and tuber dry weight of purple nutsedge among the control plants and plants inoculated with 10^2 and 10^3 conidia/ ml. However, the shoot and tuber dry weights were slightly reduced (26% for shoot dry weight and 35% for tuber dry weight) in plants treated with 10^4 conidia/ ml. The highest reductions (50% for shoot dry weight and 66% for tuber dry weight) were obtained with plants inoculated with 10^6 conidia/ ml (Fig 3-2).

Effect of Amendments on Spore Germination and Disease Development

Metamucil, N-Gel, and combinations of Metamucil or N-Gel with Silwet promoted better germination of the conidia than the control, but the percentage of spore germination was not significantly higher in Metamucil, N-Gel, or the combination of Metamucil or N-Gel with Silwet (Table 3-3). Germination of spores in the suspensions containing 0.02 % surfactants (Silwet L-77 and Triton X-100) was quite low compared to the humectants, but significantly higher than in the control (Table 3-3).

The ability of different amendments to support lesions development on purple nutsedge plants was also recorded. The humectants and the combination of the humectants with Silwet promoted more lesion development per leaf (Table 3-3). Significantly higher numbers of lesion developed on leaves sprayed with inoculum containing the humectants ($P = 0.05$, Table 3-3). Fewer lesions developed on leaves sprayed with the inoculum plus surfactants, and much fewer lesions developed on leaves sprayed with spores suspended in water (Table 3-3).

Metamucil, N-gel, and Kelzan serve as better formulation materials for *D. higginsii*. This conclusion is based on the number of diseased leaves per plant and the level of disease incidence and disease severity. Plants sprayed with conidia suspended in 0.5% Metamucil and 0.5% N-gel developed severe disease symptoms (100% disease incidence), and all leaves on these plants were severely diseased (95% and 87% disease severity, Table 3-3). Kelzan, a xanthan gum, was also a good material for formulation of this bioherbicide, but it did not perform as well as Metamucil and N-Gel. However, it was slightly better than Silwet L-77 and Triton X-100. Silwet L-77 is a commonly used

surfactants in herbicidal application. Very low levels of disease were recorded on plants spray-inoculated with the conidia suspended in water (control; Table 3-3).

Figure 3-3 illustrates the ability of the *D. higginsii* to control purple nutsedge when the conidia were formulated in 0.02% Silwet L-77, 0.5% N-Gel, or 0.5% Metamucil, treatments without amendments (conidia suspended in water), or a control (no fungus). Nearly 100% of the plants sprayed with conidia suspended in 0.5% Metamucil or N-Gel were heavily diseased 15 days after inoculation, while about 65% of the plants were killed in Silwet-amended treatments. The lesions on these leaves tended to coalesce and cause necrosis, which killed not only the leaves but also the whole plants. The lesions on plants inoculated with conidia suspended in Silwet tended to coalesce and were not distributed over the whole leaf surface. Very few plants sprayed with the water-suspended conidia developed lesions, and the lesions on these plants were confined to the original leaves that developed lesions. These lesions never spread from the diseased leaves to the others.

Effect of Dew-Period Temperature and Growth-Stage of Purple Nutsedge on Disease Development

Disease did not develop on noninoculated plants. Disease severity on inoculated plants increased as the temperature was increased during the 2 weeks following inoculation at all plant-growth stages. Disease severity reached 98% at the temperature of 25°C to 30°C on the 4- and 6-leaf-stage plants and 75% on the 8-leaf-stage plants (Fig. 3-4). Disease severity at all dew-period temperatures was significantly lower on the 8-

leaf-stage plants than on the 4- or 6-leaf-stage plants (Fig 3-4). Disease severity was significantly reduced at 35°C for all plant-growth stages.

Regression analysis of dew temperature and plant growth stage on the time to cause 50% disease severity is shown in Table 3-4. The effect of dew-period temperature and plant-growth stage were negatively correlated with the time (day) to reach 50% disease severity ($P = 0.0001$). Moreover, about 86% ($R^2 = 0.86$) of the variation in the time to reach 50% disease severity was explained by dew temperature and plant-growth stage (Table 3-4), indicating a significantly high correlation. The regression coefficient for dew-period temperature and plant-growth stage is significantly different from zero ($P = 0.0001$, Table 3-4), which explained the effect of dew-period temperature and plant-growth stage on the time required to cause 50% disease severity. At 15°C, the time required to cause 50% disease severity on all growth stages of purple nutsedge was slow (>18 days) compared to the other temperature regimes (Table 3-5). It took 10 days post-inoculation for the 4- and 6-leaf-stage plants to develop 50% disease severity, whereas 16 days for the 8-leaf-stage plants. The 4- to 6-leaf growth-stage plants were more susceptible to infection by *D. higginsii* compared to the 8-leaf stage plants.

Effect of Dew-Period Duration and Plant-growth Stage of Purple nutsedge on Disease Development

Disease developed with 0 h of dew, but at a very low level of severity, 2 wk after inoculations. Disease severity increased with increased length of dew period for all stages of plant growth (Fig. 3-5). The minimum dew period that promoted 75% disease

severity was 12 h (for the 4- to 6-leaf-stage plants) and the maximum dew-period required for maximum disease development was 24 h for the 4- to 6-leaf-stage plants (95% disease severity) and the 8-leaf-stage plants (75% disease severity, Fig 3-5). Disease development was significantly reduced for the 8-leaf-stage plants for all dew durations compared to the 4- or 6-leaf-stage plants (Fig 3-5).

Regression analysis of dew duration and plant-growth stage on the time to cause 50% disease severity is summarized in Table 3-4. Dew-period duration and plant-growth stage were highly positively correlated with time (day) to cause 50% disease severity (P = 0.0001). Nutsedge plants exposed to 6 h of dew-period took more than 18 h to develop 50% disease severity irrespective of the stages of plant-growth (Table 3-5). It took longer to develop 50% disease severity for the 8-leaf stage plants when exposed to 12, 18, 24, and 30 h of dew compared to the 4- or 6-leaf-stage plants (Table 3-5). A similar amount of time (Table 3-5) was required to cause 50% disease severity for the 4- or 6-leaf-stage plants when exposed to 12, 18, 24, and 30 h of dew. However, it took 10 days for the 4- or 6-leaf-stage plants to develop 50% disease severity and 13 days for the 8-leaf-stage plants to reach this severity level when exposed to dew periods of 24 and 30 h. Exposure of the plants to longer dew periods did not further reduce the time to cause 50% disease severity.

Discussion

To create an epidemic, one must begin with a pathogen that is capable of being virulent on the target weed and aggressive under local environmental conditions. For a

Table 3-1. Effect of conidial concentration on the development of Dactylaria leaf blight of purple nutsedge caused by *Dactylaria higginsii*.

Inoculum concentration (conidia/ml)	AUDPC[a]	r_G[b] (unit/ day)	Incubation period[c] (days)
0	0	NA[d]	NA
10^2	0	NA	NA
10^3	80.70	NA	10
10^4	976.70	0.01	6
10^5	4210.00	0.128	4
10^6	6533.30	0.147	4
10^7	3133.30	0.092	4

[a] Area under disease progress curve.
[b] Disease progress rate described by the Gompertz model.
[c] Time between inoculation and first visible symptoms.
[d] NA = Not applicable.

Table 3-2. Regression analysis of the effect of conidial concentration on shoot and tuber dry weight of purple nutsedge inoculated with *Dactylaria higggínsii*.

	Shoot dry weight	Tuber dry weight
Root MSE	1.77	1.13
C.V.	12.02	12.35
R^2	0.88	0.83
Intercept	3.25	4.04
Log conc.	-0.85	-2.70

MSE = mean square error.
C.V. = coefficient of variation.
R^2 = square of the multiple correlation.
Log conc. = log conidial concentration (slope).

Table 3-3. Effect of amendments to *Dactylaria higginsii* conidia on spore germination and disease development.

	Spore germination (percentage)[u]	Number of of lesions /leaf[v]	Number of diseased leaves/plant[w]	Disease incidence (percentage)[x]	Disease severity (percentage)[y]
Silwet	64.3b[z]	22.3c	3.8b	63.5b	59.0c
Triton X-100	63.8b	9.8b	3.0a	57.5b	40.0b
Metamucil	93.0c	36.0d	6.0c	100e	95.0f
N-Gel	92.8c	37.8d	6.0c	100e	87.0e
Kelzan S	96.0c	26.7cd	5.5c	95.5de	85.0e
Natrosol	96.0c	25.8cd	2.5a	76.8c	58.0b
Silwet + Metamucil	91.5c	33.0cd	5.6c	86.8d	90.0ef
Silwet + N-Gel	96.5c	25.5cd	2.6a	85.0d	75.0d
Silwet + Kelzan S	94.0c	20.5c	3.8b	85.0d	70.0d
Control (Water)	27.0a	4.3a	3.3a	16.3a	5.0a

[u]A spore was considered to have germinated when the length of the germ tube was twice the length of the spore.

[v]Number of lesions per leaf was assessed five days after inoculation.

[w]Number of diseased leaves per plant was assessed based on six leaves per plant, 15 days after inoculation.

[x]Number of plants that were visibly diseased relative to the total number of plants assessed.

[y]Area of plant tissue diseased divided by the total area.

[z]Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD at $P = 0.05$. Percentage values were transformed with arcsine before the analysis of variance.

Table 3-4. Regression analysis of the effect of dew-period temperature, dew-period duration, and plant-growth stage on the time taken to reach 50% disease severity of Dactylaria leaf blight of purple nutsedge inoculated with *Dactylaria higginsii*.

	Dew-period temperature	Dew-period duration
Root MSE	3.65	2.20
C.V.	16.32	16.53
R^2	0.73	0.86
Intercept	18.00	14.17
Temp	-0.30	-0.40
Growth stage	0.87	0.75

MSE = mean square error.
C.V. = coefficient of variation.
R^2 = square of the multiple correlation.
Temp = slope for temperature.
Growth stage = slope for plant-growth stage.

Table 3-5. Effect of dew-period temperature, dew-period duration, and plant-growth stage on time in days to reach 50% disease severity of purple nutsedge inoculated with *Dactylaria higginsii*.

	Purple nutsedge-growth stage		
	4-leaf-stage	6-leaf-stage	8-leaf-stage
Dew-period temperature (°C)			
15	>18	>18	>18
20	18	18	>18
25	10	10	16
30	10	10	16
35	18	18	>18
Dew-period duration (h)			
0	NA	NA	NA
6	18	18	>18
12	13.5	14	16
18	12	13	15
24	10	10	13
30	10	10	13

NA = Not applicable.
The number of days for the inoculated plants to reach 50% disease severity was determined from the disease progress curve. Data are the averages of two trials, each with four replicates.

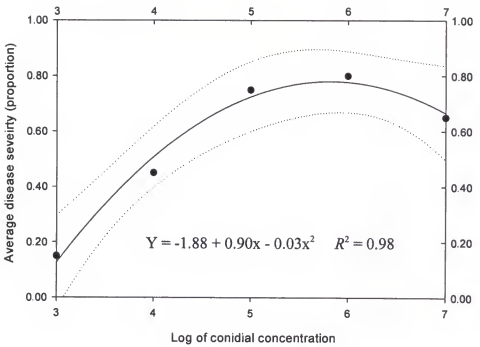

Fig. 3-1. Relationship of average disease severity from several conidial concentrations of *Dactylaria higginsii*. Plants were incubated in a dew chamber for 24 h at 28°C following inoculation and assessed for disease severity after 2 wk. Results were averaged from two experiments, each with four replicates. Curves with broken lines represent the 95% confidence interval.

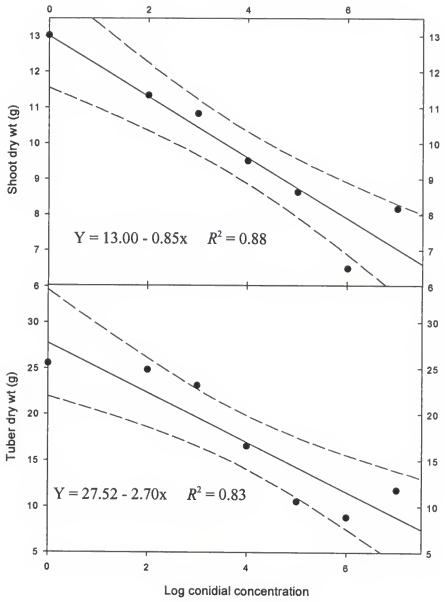

Fig. 3-2. The effect of conidial concentration on shoot and tuber dry weight (g) of purple nutsedge plants inoculated with *Dactylaria higgginsii*. Shoot and tuber dry weight were determined 45 days after inoculation. Each data point represents the mean values of two trials with four replicates each. Curves with broken lines represent the 95% confidence interval.

Fig. 3-3. Effect of conidia of *Dactylaria higginsii* suspended in different carriers on disease severity and mortality of purple nutsedge: 0.05% N-Gel + conidia (**A**), 0.02% Silwet L-77 + conidia (**B**), control, 0.5% Metamucil only (**C**), water + conidia (**D**), and 0.5% Metamucil + conidia (**E**).

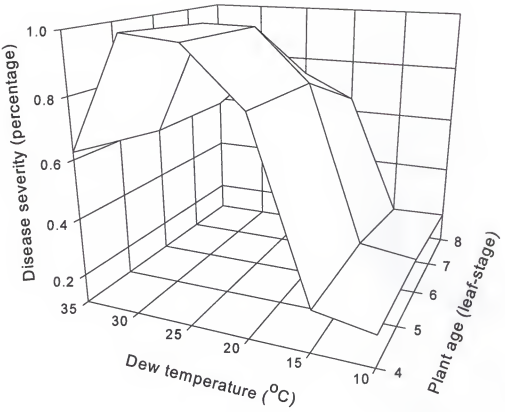

Fig. 3-4. Effects of dew temperature (°C) and plant-growth stages on the average disease severity on purple nutsedge plants inoculated with *Dactylaria higginsii*. Average disease severity values are the means of two trials, with four replicates each. Disease severity was assessed up to the days when 50% of the plants in the most effective treatment were dead.

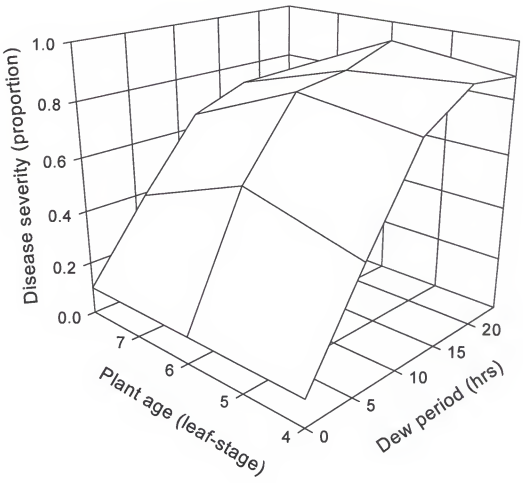

Fig. 3-5. Effects of duration of dew-period and plant-growth stage on the average disease severity on purple nutsedge plants inoculated with *Dactylaria higginsii*. Average disease severity values are the means of two trials, with four replicates each. Disease severity was assessed up to the days when 50% of the plants in the most effective treatment were dead.

polycyclic epidemic, virulence and aggressiveness of a bioherbicidal agent are linked to the maximum reproduction capacity (Shrum, 1982). Maximum reproduction is most easily facilitated by disseminating propagules as early in the growing season and as uniformly as possible across the target area. Earliness and good coverage provide several advantages: it allows for (1) the utilization of the moist and moderate weather conditions; (2) maximization of the number of infection cycles, (3) minimization of the negative impact of inoculum isolation; and (4) maintenance of the effectiveness of the agent for disease development (Shrum, 1982).

Good coverage is often linked to the large quantity of viable inoculum, which is important from two perspectives: 1) more inoculum gives better overall distribution for early development of epidemics and 2) more inoculum gives a higher initial level of disease from which successive cycles of infection develop (Shrum, 1982). The very fact that deficient inoculum is a common early-season condition in natural ecosystems provides an ideal opportunity to create the imbalance that can lead to destructive epidemics. One such opportunity is to apply a high concentration of inoculum inundatively (Charudattan, 1988a) often involving 10^6 or 10^7 conidia/ml. In the present experiment, the use of 10^6 conidia/ml applied at the rate of 100 ml/m^2 volume, which is equivalent to 10^{12} conidia in 1000 liters of water per ha (105 gallon/acre), was sufficient to give satisfactory control of purple

nutsedge. This inoculum concentration has been reported to be effective for other biological control agents (Makowski, 1993; Morin et al., 1989; Ormeno-Nunez et al., 1988; Wymore and Watson, 1986). Inoculum concentration lower than or 10-fold higher

than this level did not provide acceptable control. Low initial inoculum level resulted in fewer infections, which in turn delayed the apparent epidemic rate.

At an inoculum concentration of higher than 10^6 conidia/ml, the disease development was reduced further because spore germination was lower, due to autoinhibition occurring among the spores. This phenomenon has been illustrated by Vanderplank (1975) and demonstrated by Makowski (1993) and Heiny and Templeton (1991). It is also possible that the plant surface may compensate for this growth inhibition through interaction with chemical characteristics of the plant cuticle or dilution due to spatial distribution on plant surface (Allen et al., 1991; Makowski 1993). Another reason why concentration greater than 10^6 conidia/ml may contribute to poor control of the weed is that, at this concentration, the weeds are killed too quickly. For an aggressive weed that is propagated through underground parts, like purple nutsedge, such fast killing of the aerial parts favor an instant release of the tuber or bulb dormancy. This results in the multiple resprouting of the tubers and bulbs, and the available inoculum is not sufficient to reinfect the rapidly growing sprouts.

Nonuniformity of inoculum distribution is one of the obstacles to the efficiency of biological control agents. There are several factors that contribute to nonuniformity of inoculum distribution. Morphology and orientation of the leaves play a major part in determining the distribution of the inoculum. Purple nutsedge, which has a thick waxy upper epidermis and stomata distributed on the underside of nearly upright leaves, is not an easy target for foliar pathogens such as *D. higginsii*. This has been illustrated in this study where conidia applied in water without any amendments produced only a few

lesions per leaf, and consequently a low level of disease severity, even though the inoculum was applied at the most effective concentration (10^6 conidia/ml).

The number of initial infections by the pathogen as well as the speed of disease development and rate of secondary infections on the target weed are dependent on optimum environmental conditions (TeBeest et al., 1992). In the field, a high number of initial infections by a bioherbicide are generally essential to ensure rapid and complete weed control (Yang and TeBeest, 1992). The environmental conditions prevailing on the surface of plants are frequently hostile for biological control fungi (Andrews, 1992; Kenerley and Andrews, 1990). A requirement of more than 12 h of dew period for severe infection has been reported for several potential bioherbicide pathogens (Boyette and Walker, 1985; Makowski, 1993; McRae et al., 1988; Morin, et al., 1990; Wymore et al., 1988). An extended dew period of more than 18 h has been reported to be required by most fungal bioherbicides. The requirement of an extended dew period has been reported to be responsible for poor efficacy of many fungal weed bioherbicide efforts in the field (Charudattan, 1991; TeBeest, 1991; Watson and Wymore, 1990). However, the length of dew period required for most of the effective bioherbicides can be reduced by appropriate timing of application to take advantage of the humidity provided by rain, dew, and irrigation in the field. The formulation of foliar bioherbicides with water-retaining materials is another promising approach to make pathogens less dependent on available water to initiate infections (Daigle and Connick, 1990; Kennedy et al., 1991). Recent research on formulation has shown the potential of material such as surfactants (Zhang and Watson, 1997; Walker and Riley, 1982; Walker, 1981), invert emulsions (Amsellen

et al., 1991; Daigle et al., 1989), oils (Egley and Boyette, 1994), humectants, and polymeric gel (Boyette et al., 1996) to overcome dew requirements , which will broaden the application strategies for bioherbicides. Shabana et al. (1997) demonstrated that 0.5% Metamucil and polymeric gels promoted germination of conidia and subsequent disease development of *Alternaria cassiae* and *A. eichhorniae*. Their findings that 0.5% Metamucil and N-Gel are the best materials to formulate the inoculum of *Alternaria* spp. were corroborated by the results from this study. The ability of the inoculum of *D. higginsii* to cause high levels of disease severity and subsequent death of purple nutsedge was promoted by Metamucil and N-Gel. Other amendments, such as the surfactant, only provided marginal control.

The temperature during the dew-period is another important factor that determines the success of disease development (McRae and Auld, 1988; Heiny and Templeton, 1993; Makowski, 1993). Usually the effect of the dew-period required and the temperature typically interact. The optimum dew-period temperature for the *D. higginsii*-purple nutsedge pathosystem was in the range of 20°C to 30°C. This temperature range has been reported as being suitable for other bioherbicides, except *Colletotrichum gloeosporioides* f. sp. *malvae*, a bioherbicide to control *Abutilon theophrasti* Medik., which required a temperature near 15°C for effective control (Makowski, 1993). *Colletotrichum orbiculare* (Berk. and Mont.) v. Arx. required 35°C and a dew-period of 8 h for effective control of *Xanthium spinosum* L. (McRae and Auld, 1988). The range within which *D. higginsii* is most effective coincides with the temperature in the early cropping seasons in the warm, temperate regions. A temperature of above 30°C renders *D. higginsii*

ineffective to control purple nutsedge, because under hot temperatures, purple nutsedge grows actively and reaches maturity rapidly (Bendixen and Nandihalli, 1988). The available inoculum, without additional secondary inoculum, cannot match the fast growth of purple nutsedge. Therefore, the application of *D. higginsii* too early or too late in the growing season is not desirable, because it is not very effective at temperatures below or above the conducive temperature range (15 °C - 30°C).

Even though all growth stages of the weed were susceptible, the younger, 4- to 6-leaf-stage plants were more susceptible at 20°C to 30°C and with a minimum dew duration of 12 h compared to the 8-leaf-stage plants at the same dew-period temperature and dew-period duration. The susceptibility of the younger seedlings to other bioherbicidal agents has been reported (Boyette and Walker 1985; Charudattan 1988a; TeBeest et al., 1991; Zhang and Watson 1997). On the other hand, Makowski (1993) and Morris (1983), reported that older plants of round-leaf mallow (*Malva pusilla* Smith) and *Hakea sericea* Schrad. were more susceptible to *Colletotrichum gloeosporioides* f. sp. *malvae* and *Colletotrichum gloeosporioides*, respectively. They explained the decrease in susceptibility of younger plants as being due to the ability of the actively growing tissue to partially outgrow the disease.

Thus *D. higginsii* has the epidemiological characteristics of a potentially effective bioherbicide. It is highly virulent, has tolerance to a broad range of environmental conditions, and this epidemiological range corresponds to the range in which this weed is a problem.

CHAPTER 4
EFFECT OF *Dactylaria higginsii* ON PURPLE NUTSEDGE INTERFERENCE WITH TOMATO AND PEPPER

Introduction

Purple nutsedge has been reported to cause a high percentage of yield loss in vegetable crops through interference. William and Warren (1975) reported that the presence of purple nutsedge in transplanted tomato in Brazil could cause as much as 53% yield loss. In the United States, Keeley (1987) reported that yield loss due to interference by purple nutsedge in garlic could reach as high as 89%; okra, 62%; carrot, 39-50%; cucumber, 43%; and cabbage, 35%. Although chemical herbicides have provided a fair degree of control, environmental factors and plant-growth stages have been reported to account for poor control (Gricher et al., 1992). Several other nonchemical methods have been used, but none have provided acceptable control. Long-term, sustained control of purple nutsedge has been difficult to achieve.

The use of a bioherbicide to reduce interference by purple nutsedge in cropping systems has not been reported because research into this area has only started recently. However, there are several reports of success in reducing weed interference in different cropping systems with the help of biological control agents. For example, Pantone et al. (1989a; 1989b) reported that the addition of fiddleneck flower-gall nematode [*Anguina amsinckiae* (Steiner and Scott) Thorne] to mixtures of wheat and coast fiddleneck (*Amsinckia intermedia* Fisher and Mayer) greatly increased the ability of wheat (*Triticum*

aestivum L.) to suppress the ability of coast fiddleneck to flower and set seed. The addition of the nematode reduced fiddleneck's negative effect on wheat and increased wheat seed yield. Kennedy et al. (1991) conducted laboratory and field studies to examine the effects of *Pseudomonas fluorescens* Migula strain D7 on downy brome (*Bromus tectorum* L.), is a major pest in winter wheat in northwestern United States. They reported that the application of this rhizosphere bacterium reduced germination, biomass production, and seed production of downy brome, but did not have any inhibitory effects on wheat. The suppressive effect of *P. fluorescens* strain D7 was attributed to the production of phytotoxin (Tranel et al., 1993; Gurusiddaiah et al., 1994). Paul and Ayers (1987), who conducted field experiments with lettuce (*Lactuca sativa* L.), groundsel (*Senecio vulgaris* L.), and a rust pathogen (*Puccinia lagenophorae* Cooke) which infects groundsel, reported that the fresh yield of lettuce was significantly inhibited by competition from noninfected groundsel. Yield was reduced at sowing densities from 250 to 65,000 seeds/m^2, but was not reduced by the rust-infected groundsel until weed density reached 4000 seeds/m^2. Lettuce yield in plots containing diseased groundsel was two or three times greater than in plots in which the weed was noninfected by the pathogen. Jacobs et al. (1996), reported that *Sclerotinia sclerotiorum* (Lib.) de Bary was able to decrease competition between bluebunch wheatgrass [*Agropyron spicatum* (Pursh.) Scribn. and Smith] and spotted knapweed (*Cantaurea maculosa* Lam.). The height of blue bunchgrass was 3.5 times greater than that of spotted knapweed. The coefficient ratios (which estimate the interaction between the two plants) were 2.11 (bluebunch wheatgrass) and 0.51 (spotted knapweed), which indicate a greater influence of bluebunch wheatgrass on knapweed than of knapweed on bluebunch wheatgrass. They

also reported that *S. sclerotiorum* reduced spotted knapweed density by 68 to 80% without reducing bluebunch wheatgrass density. Spotted knapweed weight per plant also was reduced by the addition of *S. sclerotiorum*.

Dactylaria higginsii, a fungus isolated from diseased purple nutsedge has been reported to be capable of controlling this weed (Kadir and Charudattan, 1996; Kadir et al., 1997a; 1997b). However the potential to apply this bioherbicidal agent in cropping systems to reduce interference from purple nutsedge has not been studied. Hence, the objectives of this research were: 1) to determine the effect of *D. higginsii* on the interference of purple nutsedge on tomato and pepper and 2) to determine the effective inoculum concentration needed to reduce interference from purple nutsedge.

Materials and Methods

Experimental Method

The experimental method used in this study was the additive series approach. In this method, the density of one species (usually, called the indicator crop) is held at a constant and the density of the other species (the weed) is varied. Since the latter is added into the first of this bipartite series, this approach is called the additive series. This system uses the response of the first species in fixed density as an indicator of the relative aggressiveness or competitive ability of the second species to the first. This system is applicable in cropping systems with encroaching weeds and in intercropping systems (Cousens, 1990; Nickel et al., 1990). The additive system has a universal phenomenon incorporating the Law of Diminishing Returns. The increasing density of the second species into the area of the first species reduces the yield of the first species at an

increased rate. This occurs until further increase in the density of the second species does not result in further decrease of the yield of first species .

Interference

The experiment was carried out in two greenhouses in spring 1996 and repeated in fall 1996, using transplants tomato cv Agroset and pepper cv Capistrano (obtained from Dr. W. M. Stall, Hort. Science Dept., University of Florida, FL.) as indicator crops. The mixture of tomato or pepper and purple nutsedge were grown in 30-cm (diam) x 10-cm (height) pots filled with 0.07 m^3 of commercial potting medium (Metro Mix 220, Scott-Sierra Horticultural Product Co., Maryville, OH.) consisting of horticultural vermiculite, Canadian spaghnum peat, and horticultural perlite. Each pot was planted with one transplant of tomato or pepper and one of the following purple nutsedge densities: 0, 40, 80, 160, and 320 tubers per m. After transplantation, the soil moisture was kept at field capacity by drip irrigation three times daily. Soil fertility was maintained by adding water-soluble Peters Professional All Purpose Plant Food (20:20:20 + Trace Elements, Spectrum Group, Div. of United Industries Corp., St Louis, MO) at the recommended rate of 3.785 liters of solution (9.5 g/3.785 liters water) for 0.09 $meter^2$ bed, every two weeks.

Fungal Inoculation

Inoculum used in this experiment was produced in trays on a thin layer of PDA (see Chapter 3). Three inoculum concentrations were used: 0 [0.5% Metamucil (w/v), used as a humectant; as a control]; 10^4 conidia/ml with 0.5% Metamucil (w/v); and 10^6

conidia/ml with 0.5% Metamucil (w/v). One hour before the plants were to be inoculated, they were misted for 5 min to wet the leaf surfaces. The plants were inoculated by spraying the conidial suspension with an aerosol sprayer until runoff. Starting 6 h after inoculation, the greenhouse misters were turned on for 5 min at every 6-h interval for the first 24 h to maintain leaf wetness. This was to ensure that *D. higginsii*, which requires a dew-duration period of at least 12 h for disease development (Chapter 3), would be able to infect purple nutsedge under greenhouse conditions.

Data Collection

Disease severity was assessed every five days using the modified Horsefall Barratt scale (see chapter 2) (Horsefall and Barratt, 1945). Disease progress curves were obtained for each treatment and the area under the disease progress curve (AUDPC) was calculated for each treatment. Tomato and pepper were harvested at 50 and 65 days after transplantation. The yield was recorded as the number of fruits and fruit weight per plant. The crops were harvested twice, because the yields during the first harvest did not show any expected trend. The data from the first and second harvest were pooled and recorded as the total yield per plant. Tuber numbers were recorded at the final harvest time (65 days after transplantation), from each pot. The tubers and the bulbs were separated after washing the soil from the roots and rhizomes. Both were recorded as tubers. Shoot and tuber biomass were determined at harvest time by weighing the shoots and tubers after they were dried at 75°C for 5 days.

Data Analysis

The study was carried as a factorial experiment with two factors (tuber densities as the main factor and inoculum concentration as the sub-factor) each for tomato and pepper. The experiment had a randomized complete block design with four replicates. The factorial analysis of the experiment considered the effect of each factor individually as well as their interactions. Results from the two trials of each experiment were pooled if homogeneity of variances was confirmed by Barlett's test (Gomez and Gomez, 1984). Mean values of four replications were used for statistical analysis and the treatment means were separated with Fisher's protected LSD at the 5% significance level. Orthogonal contrasts of the log inoculum concentration and tuber densities, and of the slopes of the linear regression models, were performed to determine the individual effect of tuber density and inoculum concentration and their interactions on weed-growth components and crop yields.

Results

The homogeneity of variance among treatments was confirmed by Barlett's test (Gomez and Gomez, 1984). Similar trends were noted in the levels of control of weed-growth components and disease severity of Dactylaria leaf blight on inoculated purple nutsedge in both experiments from both trial dates. The data on weed growth and disease severity, which were expressed as area under the disease progress curves (AUDPC) were averaged over all trials dates and over both indicator crops (tomato and pepper).

Effect of *Dactylaria higginsii* on Weed-Growth Components

Orthogonal contrasts for the effect of log inoculum concentration of *D. higginsii* with various tuber densities showed highly significant quadratic relationship ($P< 0.001$) on weed -growth components. Likewise, the orthogonal contrasts for the effect of initial planting densities of tubers with the control and treatments inoculated with 10^4 conidia/ml showed a highly significant quadratic effect ($P< 0.001$) on weed-growth components, except for 10^6 conidia/ml (Table 4-1).

Based on the orthogonal contrast (above), the relationship between the final weed-growth components with tuber density was best explained by the 2nd order polynomials (Fig. 4-1). The initial planting density of tubers had a significant effect on all final weed-growth components of purple nutsedge in noninoculated control and in treatments where plants were inoculated with 10^4 conidia/ml. The final weed-growth components of purple nutsedge increased with increasing purple nutsedge tuber density, and the relationship was initially linear and peaked at 160 tuber per m^2, after which the increase was no longer linear. Exception was the treatment in which the purple nutsedge plants were inoculated with *D. higginsii* at 10^6 conidia/ml. The final weed-growth components were not affected by the initial planting density of tubers. (Fig. 4-1). The final weed-growth components were significantly reduced in treatments where purple nutsedge plants were inoculated with *D. higgginsii* at 10^6 conidia/ml regardless of initial planting densities of tuber. This reduction was significant when compared to the noninoculated weedy control as well as the treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml (Fig. 4-1).

The comparison of the slopes for quadratic relationship (Appendix A-2, A-3, and A-4 for the equation) of growth components of purple nutsedge with initial planting densities of tubers are shown in Table 4-2. The slopes of the noninoculated control and treatments of purple nutsedge plants inoculated with 10^4 conidia/ml were similar ($P > 0.05$), but the slopes of treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml were significantly lower ($P = 0.001$) compared to the noninoculated control and 10^4 conidia/ml. Thus, the growth of purple nutsedge was suppressed in treatments where plants were inoculated with 10^6 conidia/ml.

Effect of *D. higginsii* on AUDPC

Purple nutsedge plants inoculated with 10^4 conidia/ml developed low levels of disease compared to plants inoculated with 10^6 conidia/ml (Fig. 4-2). Most of the disease that developed on plants inoculated with 10^4 conidia/ml was confined to the margins and tips of the leaf laminas. Lesion expansion did not compensate for the abundant resprouting of the diseased plants, thus very low levels of secondary infections occurred in treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml. The amount of disease was high in treatments where purple nutsedge plants were inoculated with 10^6 conidia/ml, and almost all of the plants in this treatment died (Fig. 4-2). Very little regrowth was observed in treatments where purple nutsedge plants were inoculated with 10^6 conidia/ml, and secondary spread from the previously diseased leaves caused subsequent infection on the regrowth.

The disease severity of the inoculated plants was expressed as the AUDPC (Table 4-3). The AUDPC values were not affected by tuber densities (Table 4-3) in both

experiments (pepper and tomato) and at both inoculum concentrations. The AUPDC values of treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml were lower compared to AUDPC values of treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml. The apparent infection rates (r_G) of the treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml (r_G = 0.046 - 0.050) was slower compared to the apparent infection rates (r_G = 0.126 - 0.136, Table 4-3) of purple nutsedge plants inoculated with10^6 conidia/ml.

Effect of *Dactylaria higginsii* on Tomato and Pepper Yields

The effect *of D. higginsii* on yields of tomato and pepper was best illustrated by the yield-loss equation, $Y_L = aN_w/ (1 + aN_w/m)$ (Cousens, 1985) where:

Y_L is relative yield loss (percentage)

N_w is weed density (plants/m^2)

a is the parameter that describes the effect of adding the first weed (m^2/plant)

m is the maximum yield loss of less than 100%.

Initial planting densities of tubers had a significant effect on the percentage yield loss of tomato and pepper for both the noninoculated controls and the treatments with 10^4 conidia/ml. Orthogonal contrasts for the effect of log concentration of *D. higginsii* with various tuber densities showed highly significant quadratic effects ($P< 0.001$) of *D. higginsii* on the percentage yield loss of both crops (pepper and tomato), except the weed-free control (Table 4-4). Similarly, the orthogonal contrasts for the effect of initial planting densities of tubers with the control and 10^4 conidia/ml showed a highly

significant quadratic effect ($P < 0.001$) on the percentages of yield loss, except for 10^6 conidia/ml (Table 4-4).

Based on these contrasts, the relationship between the initial planting densities of tubers and the percentages of yield loss was best explained by the 2nd order polynomials (Fig. 4-3). The percentages of yield loss for both pepper and tomato increased linearly with increasing purple nutsedge tuber density, and peaked at 160 tuber per m^2. An exception was the treatments in which the purple nutsedge plants were inoculated with *D. higginsii* at 10^6 conidia/ml. The percentages of yield loss were not affected by the initial planting density of tubers (Fig. 4-3). The percentage of yield loss was significantly reduced in treatments where purple nutsedge plants were inoculated with *D. higggínsii* at 10^6 conidia/ml regardless of the initial planting densities of tubers compared to the noninoculated weedy control and treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml (Fig. 4-3).

The percentage of yield loss of pepper was significantly higher even at 40 tubers/m^2 (19.07% for the control and 17.42% for 10^4 conidia/ml) compared to the percentage of yield loss of tomato at the same tuber density (3.54% and 4.32% for the control and 10^4 conidia/ml, respectively Fig. 4-3). The percentage of yield loss was significantly higher in pepper compared to tomato at all tuber densities (Fig. 4-3). However, the percentages of yield loss for both pepper and tomato in treatment with 10^6 conidia/ml were significantly lower, even with the highest tuber density (4% in pepper and 2% in tomato Fig. 4-3). The percentages of yield loss of pepper and tomato among tuber densities within the 10^6 conidia/ml treatments were not significantly different (Fig. 4-3).

The comparison of the slopes for quadratic relationship (Appendix A-1 for the equation) of percentages of yield loss with initial planting densities of tubers are shown in Table 4-5. The slopes of the noninoculated control and treatment inoculated with 10^4 conidia/ml were similar ($P > 0.05$), but the slopes of the treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml were significantly less compared to the noninoculated control and 10^4 conidia/ml. This meant that the growth of purple nutsedge was suppressed in the treatment where the plants were inoculated with 10^6 conidia/ml (Fig. 4-4).

Discussion

Dactylaria higginsii did not infect pepper or tomato. This was expected as this fungus had been previously determined to be host specific to *Cyperus* spp. Infection was observed on purple nutsedge in the control, due to cross-contamination, but the level of infection was below 5% severity. This level was very low to account for any significant effect on the yield of crops or the growth components of purple nutsedge.

The effect of initial tuber densities on the growth components of purple nutsedge were linearly correlated, but reached the saturation point at 160 tubers/m^2. Beyond this point, further addition of the tubers did not significantly increase the growth components of the weed. William (1981) and William et al. (1977) also found that the tuber weight of yellow nutsedge (*Cyperus esculentus* L.) did not increase with interspecific competition at higher tuber densities, either on an individual plant basis or stand basis. Likewise, in the experiments inoculated with 10^4 conidia/ml the tuber weight of purple nutsedge did not increase. In fact, all weed-growth components in the treatment with 10^4 conidia/ml.

Table 4-1. Orthogonal contrasts of log inoculum concentration of *Dactylaria higginsii* and tuber densities on growth of purple nutsedge.

	Crop: Pepper					
Contrast	Shoot dry weight Mean (g)	F-value	Tuber dry weight Mean (g)	F-value	Final tuber number Mean (no.)	F-value
Quad log conc., td[a]= 40	23.13	129.25***	31.27	128.21***	52.96	4.38***
Quad log conc., td = 80	66.63	1115.02***	72.38	643.17***	115.50	288.95***
Quad log conc., td = 160	74.60	1080.20***	77.15	665.58***	119.17	308.47***
Quad log conc., td = 320	90.42	1538.85***	84.21	889.00***	128.37	432.06***
Quad td, conc. = 0	91.97	775.77***	96.42	803.95***	151.28	386.25***
Quad td, conc. = le4	94.66	692.60***	96.68	692.60***	155.10	297.62***
Quad td, conc. = le6	4.26	0.82NS	5.63	1.38NS	5.63	0.18NS
	Crop: Tomato					
Quad log conc., td[a]= 40	47.96	224.85***[c]	25.42	93.96***	45.00	64.88***
Quad log conc., td = 80	52.67	502.41***	66.50	502.41***	100.04	265.36***
Quad log conc., td = 160	112.62	1065.95***	70.67	632.79***	102.71	258.01***
Quad log conc., td = 320	113.67	1146.26***	74.63	644.85***	104.84	279.63***
Quad td, conc. = 0	121.16	602.97***	88.44	656.97***	132.29	398.68***
Quad td, conc. = le4[b]	118.69	569.79***	86.82	649.92***	125.54	340.40***
Quad td, conc. = le6	5.85	1.41NS[d]	2.66	0.39NS	6.63	0.86NS

[a]td = tuber density/m^2.
[b]le = log concentration.
[c]***$P = 0.001$.
[d]NS = Not significant.
Mean values are the averages of two trials, each with four replicates.

Table 4-2. Slope values and comparisons of slopes from linear regression of growth components of purple nutsedge and the initial tuber densities of purple nutsedge in pepper and tomato recorded 65 days after inoculation with *Dactylaria higginsii*.

Treatment	Crop: Pepper			Crop: Tomato		
	Shoot dry weight (g)	Tuber dry weight (g)	Final tuber number	Shoot dry weight (g)	Tuber dry weight (g)	Final tuber number
Control	1.071	1.198	2.704	1.427	1.109	2.367
10^4 conidia/ml	1.110	1.138	2.520	1.349	1.091	2.187
10^6 conidia/ml	0.046	0.057	0.045	0.086	0.030	0.116
			Contrasts			
Control vs 10^4	NS[y]	NS	NS	NS	NS	NS
Control vs 10^6	***[z]	***	***	***	***	***
10^4 vs 10^6	***	***	***	***	***	***

[z] *** = $P < 0.001$.

[y] NS = Not significant.

Table 4-3. The area under the disease progress curve (AUDPC) and the apparent infection rate (r_G) of *Dactylaria higginsii* when applied at different concentrations and with different initial densities of purple nutsedge tubers.

Densities (tuber /m^2)	Inoculum concentration (conidia/ml)	Pepper		Tomato	
		AUDPC[x]	r_G[y]	AUDPC	r_G
40	10^4	1456.4	0.049	1444.3	0.046
80	10^4	1452.5	0.047	1436.8	0.048
160	10^4	1447.1	0.050	1458.1	0.051
320	10^4	1442.3	0.049	1430.3	0.050
40	10^6	5647.5	0.113	5975.0	0.126
80	10^6	5887.5	0.112	6042.5	0.128
160	10^6	5975.0	0.117	6004.7	0.127
320	10^6	5962.5	0.123	6032.5	0.136

[x] AUDPC was calculated for disease severity from the means of two trials, each with four replicates.
[y] Apparent infection rate was calculated by using the Gompertz model.

Table 4-4. Orthogonal contrasts of the effect of log inoculum concentration of *Dactylaria higginsii* and initial tuber densites of purple nutsedge on the yield of pepper and tomato fruits.

Contrast	Crop: Pepper		Crop: Tomato	
	Mean (g/plant)	F-values	Means	F-value
Quad log conc., td[a] = 0	324.84	2.15NS[c]	1441.71	0.18NS
Quad log conc., td = 40	281.84	5.94*[d]	1385.29	0.07NS
Quad log conc., td = 80	194.71	62.54***[c]	985.29	74.91***
Quad log conc., td = 160	165.63	99.34***	956.28	70.08***
Quad log conc., td = 320	170.34	104.30***	960.21	58.57***
Quad td, conc. = 0	182.86	104.33***	1007.65	65.19***
Quad td, conc. = 1e4[b]	178.38	83.27***	1016.88	97.44***
Quad td, conc. = 1e6	321.35	1.09NS	1412.80	0.61NS

[a]td = tuber density/m^2.
[b]le = log concentration.
[c]NS = Not significant.
[d]* = $P < 0.05$.
[e]*** = $P < 0.001$.

Table 4-5. Slope values and comparisons of slopes from linear regression of percentages of yield loss of pepper and tomato on initial tuber densities of purple nutsedge recorded 65 days after inoculation with *D. higginsii*.

Treatment	Crop: Pepper	Crop: Tomato
Control	0.751	0.524
10^4 conidia/ml	0.736	0.562
10^6 conidia/ml	0.061	0.021
	Contrasts	
Control vs 10^4	NS[y]	NS
Control vs 10^6	***[z]	***
10^4 vs 10^6	***	***

[y]NS = not significant.
[z] *** = $P < 0.001$.

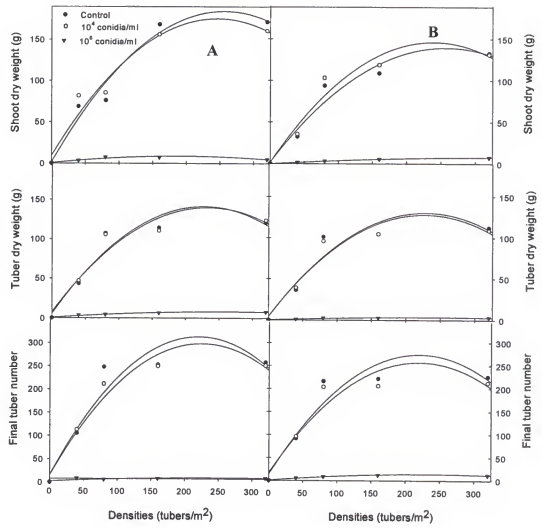

Fig. 4.1. Effect of *Dactylaria higginsii* on different growth components of purple nutsedge planted at different tuber densities. Pepper (**A**) and tomato (**B**). Each data point represents the mean values of four replicates.

Fig. 4-2. Reaction of purple nutsedge plants inoculated with *Dactylaria higginsii*. Weedy control (**A**), weed-free control (**B**), inoculated with 10^4 conidia/ml (**C**), and inoculated with 10^6 conidia/ml(**D**).

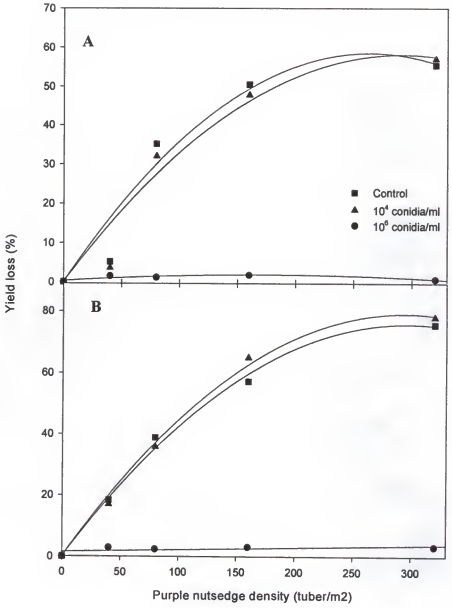

Fig. 4-3. Effect of inoculation of purple nutsedge with *Dactylaria higginsii* on the percentage yield loss of pepper and tomato. Tomato (**A**) and Pepper (**B**). Each data point represents the mean value of four replicates.

were relatively higher compared to the noninoculated control. At 10^4 conidia/ml, the level of disease was low and it was not enough to slow the fast growth of purple nutsedge, and the partial stress imposed by the low disease level merely caused the plants to increase biomass partitioning. Marambe (1996) also found the increase in biomass of partially defoliated purple nutsedge. He attributed this increase to increase in regeneration of the plants. He explained this phenomenon as the ability of the partially defoliated leaves to photosynthesize from their available leaf areas.

When the plants were inoculated with 10^6 conidia/ml, all growth components of purple nutsedge were significantly reduced irrespective of the initial planting densities of tubers. The disease levels, as determined by the AUDPC values for all tuber densities, were relatively high in treatment with 10^6 conidia/ml compared to treatment with 10^4 conidia/ml. In treatment with 10^4 conidia/ml, even though the plants were infected, the level of disease was not high enough to kill the plants, and the available inoculum was not adequate to cause sufficient infection on the rapidly growing purple nutsedge plants

Thus, purple nutsedge recovered, as indicated by the high dry weight of shoots and tubers. At 10^6 conidia/ml, the plants were killed, and the high level of initial inoculum resulted in a higher level of secondary inoculum. As a result of severe disease, there was severe secondary infection of the regrowth and the amount of regrowth was suppressed. This finding is contradictory to the report by Marambe (1996) who found that purple nutsedge, even when completely defoliated, tends to increase shoot regrowth and tuber numbers. However, he did the experiment without any crop, as was done in this study, where the presence of tomato and pepper was a significant factor. The shading

provided by the crop plants probably helped to maintain humid conditions and to promote disease development with severe secondary infection about 40 days after inoculation.

The apparent infection rate was much faster in treatments inoculated with 10^6 conidia/ml as compared to 10^4 conidia/ml. This could be explained by the presence of tomato or pepper plants that shaded purple nutsedge plants, predisposing them to infection by *D. higginsii*. The high humidity under the crop canopy provided a conducive environment for disease development. Moreover, shading appeared to have caused purple nutsedge to produce thinner and weaker shoots. Bantillan et al. (1974), Santos et al. (1997a), Peterson (1982), Sierra (1969), and William et al. (1977) found that shading reduced the light available to parent purple nutsedge plants. Thus, thinner and weaker shoots were produced that were less competitive and more prone to infection by *D. higginsii*.

The percentages of yield loss of tomato and pepper increased with increases in tuber densities of purple nutsedge, but levelled off after reaching 160 tubers/m^2. The application of *D. higginsii* at 10^4 conidia/ml did not have any significant effect in reducing the percentages of yield loss of pepper and tomato. The percentage of yield loss of pepper was significantly greater even at 40 tubers/m^2 compared to the weed-free control. At the same tuber density, even without *D. higginsii*, the percentages yield loss of tomato was not significantly different compared from the weed-free control. This implies that pepper was more sensitive to the presence of purple nutsedge even at a low density compared to tomato. The percentages of yield loss of tomato and pepper were significantly reduced irrespective of tuber densities, when purple nutsedge plants were

inoculated with 10^6 conidia/ml. This could be explained by the reduction in all weed-growth components by *D. higginsii* (explained earlier). This finding could be supported by the positive linear relationship of AUDPC values with yield; i.e., as the AUDPC values increased, yield of tomato and pepper also increased, but the rate of increase was much faster in tomato compared to pepper.

Dactylaria higginsii has the potential to reduce the interference of purple nutsedge in a cropping system. The ability of this fungus to reduce weed interference was evident when *D. higginsii* was applied at 10^6 conidia/ml. However, the field efficacy of this fungus under different cropping systems needs to be studied further.

CHAPTER 5
FIELD EFFICACY OF *Dactylaria higginsii* AS A BIOHERBICIDE FOR THE CONTROL OF PURPLE NUTSEDGE

Introduction

As explained previously, the possibility of using *Dactylaria higgginsii* as a bioherbicide to control purple nutsedge was investigated under greenhouse conditions and it was determined that this fungus was highly pathogenic to this weed (Kadir and Charudattan, 1996). The fungus is host-specific to *Cyperus* spp.; plants outside this genus are immune. Thus, this fungus has excellent potential to be used to control purple nutsedge. In greenhouse experiments, *D. higginsii* suppressed the growth of purple nutsedge and reduced the interference of this weed with tomato and pepper when applied at 10^6 conidia/ml. Based on this information, it was decided to determine the efficacy of this pathogen in the field.

Charudattan (1988b) defined bioherbicidal efficacy as the level of weed control, the speed of weed control, and the ease with which a bioherbicide can be used. These three factors are important criteria to select and evaluate a bioherbicide. Several pathogens have been tested in the field for their field efficacy with highly impressive results. For example, Charudattan (1985) and TeBeest and Templeton (1985) reported that DeVine® (*Phytophthora palmivora* (Butler) Butler) provided excellent control of milkweed vine (*Morrenia odorata* (Hook. & Lindl.)) within 4 to 6 wk when applied as postemergent aerial sprays and Collego® (*Colletotrichum gloeosporioides* (Penz.) Sacc. f.

sp. *aeschynomene*) provided greater than 85% control of northern jointvetch (*Aechynomene virginica* [L.] B.S.P.). Phatak et al. (1985) demonstrated that *Puccinia canaliculata* Lagerh. reduced the stand of yellow nutsedge by 46% and tuber formation by 66%, when this rust was applied early in spring. However, the control of yellow nutsedge was enhanced (greater than 99% control) when the rust was applied with paraquat (1, 1'-dimethyl-4, 4'-bipyridinium ion). Imaizumi et al. (1997), demonstrated that the efficacy of Camperico® (*Xanthomonas campestris* Migula pv. *poae*) to control annual bluegrass (*Poa annua* L.) in golf courses was temperature-dependent. A day temperature of 25°C and night temperature of 20°C were required. The bacterium, when applied at the rate of 400 ml/m^2 with an inoculum dose of 10^8 cfu/ml provided 100% control of this weed. However, the bacterium caused severe infection on zoysia (*Zoysia tenuifolia* Willd. Ex Trin.), a popular grass in the golf courses, when it was applied at the rate of 10^9 cfu/ml. It did not infect creeping bent grass (*Agrostis stolonifera* L.) and Kentucky bluegrass (*Poa pratensis* L.), the two most common turf grasses. Long-term control of annual bluegrass could be achieved by repeated application of this bacterium. Zhang and Watson (1977) tested *Exserohilum monoceras* Leonard & Suggs. for the control of *Echinocloa* spp. in rice by applying the pathogen either in an oil emulsion or as a dry powder at different inoculum rates. A maximum level of control (90% mortality) of this weed was achieved when the pathogen was applied at 5.0 x 10^7 conidia/m^2. The type of formulation did not have any significant effect on the level of control.

The objectives of the present investigation were to test the field efficacy of *Dactylaria higginsii* and to 1) determine the effective spore concentration needed to control purple nutsedge in the field and 2) determine the number of inoculations required to obtain significant reduction in the number and weight of purple nutsedge tubers.

Materials and Methods

Inoculum Production

Inoculum of *D. higginsii* was produced on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) amended with 3.7 mg/ml streptomycin sulfate and 2.5 mg/ml chloramphenicol. A conidial suspension collected from 14-day-old plate-culture (a concentration of 10^5 conidia/ml) was sprayed onto 300 ml of PDA in a food tray (34 cm x 42 cm x 3 cm depth). The tray was covered with sterile plastic sheets and incubated at 28°C under 12 h darkness and 12 h light. Conidia were collected after a 14-day incubation period. The agar surface was flooded with 150 ml of sterilized water, scraped with a rubber spatula, and the surface rinsed with 50 ml of sterilized water to make the final volume of 200 ml/tray. The resulting suspension was then passed through a layer of cheese cloth and the final conidial concentrations were determined with a hemacytometer. A final conidial suspension from each tray typically contained 1.5 to 2.8 x 10^6 conidia/ml.

Field Trials

Field trials were done in spring through summer of 1996, in two field locations in Florida. The trials were spatially repeated, with the first trial being done at the

Horticultural Experimental Unit, Gainesville, Florida and the second trial at the West Florida Research and Education Center, Jay, Florida. The two locations were separated by about 400 miles. Natural populations of purple nutsedge were used in both locations. The plots were first tilled and Dual® 8E (metolachlor; 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxyethyl) acetamide) and Poast® (sethoxydim; 1-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl] -3-hydroxy-2-cyclohexen-1-one) were applied at the recommended rates as preemergence and postemergence herbicides, respectively, to control broadleaf and grassy weeds.

The experiment was conducted as a 3 x 3 factorial in a randomized complete block design. This experimental design was chosen to compensate for the topography of the sites. A plot size of 1m x 1m were used. Each treated plot was separated by a 1m x 1m border grown with purple nutsedge. The treatments were: carrier (0.5% Metamucil) as control, 10^5 conidia/ml + carrier, and 10^6 conidia/ml + carrier; applied at the rate of 100 ml/m^2 as single, double, and triple postemergence sprays. The multiple applications were made at biweekly intervals. These conidial concentrations equaled 10^{11} and 10^{12} conidia in 1000 liters of water/ha (105 gallons/acre). Purple nutsedge plants were at the 4- to 6-leaf stage when inoculated.

Data Collection

Disease was assessed as disease severity based on a modified Horsefall and Barratt scale (Horsefall and Barratt, 1945; see chapter 2) at 5-day intervals for the duration of the experiments. The weed-growth components of shoot and tuber dry weight

and tuber number were collected at the end of the experiments. All shoots were cut at the soil line within the 1-m^2 plot. Tubers were obtained from 3 subsamples collected from the center of each plot. Each subsample was 4072 cm^3 in volume, taken with a tree planter, 16 cm deep and 18 cm diameter. Tubers were cleaned off the roots and soil and dried at 70°C for 7 days before dry weights were obtained.

Data Analysis

All percentage data were transformed by arcsine before analysis (Gomez and Gomez, 1984). Analysis of variance (ANOVA) using the General Linear Models and the mixed procedures were used where appropriate (SAS Institute, Cary, NC) to analyze the effect of each factor individually and their interactions. The effects of inoculum concentration and inoculation frequencies were determined by analysis of variance of the area under the disease progress curve (AUDPC) with the mixed procedure of the SAS.

Results

Location of the trial had no significant effect on the weed-growth components of purple nutsedge with the exception of shoot dry weight (Table 5-1). The presence of significant site-variance indicated that the physical characteristics and environmental differences (such as temperature and rainfall; Appendix C-1 and Appendix C-2) between the sites contributed to the differences in the shoot dry weight. The main and interaction effects of inoculum concentration and inoculation frequencies were all highly significant for the site term in the mixed model. Therefore, the conclusion is that even though there

were differences in shoot dry weight among the two sites, which contributed significantly to the results, these differences were properly compensated in the mixed model to allow comparisons among treatments as if there were no site effects. The presence of highly significant effects and interactions may occur regardless of the site, if that site is similar in characteristics to one of the two sites actually used. These highly significant effects and interactions show evidence of consistency of treatment effects over multiple sites. Consequently, the data were combined and reported as combined results.

Secondary disease spread was visible in the border rows and in the control plots, which were not protected with a fungicide. However, the disease severity in the control treatment never reached 5% and there was no significant effect on the growth-components of purple nutsedge. Disease level was initially low in plots inoculated with 10^5 conidia. It remained low throughout the experiment. Disease progress for this rate was very slow in these plots. Disease level never reached 50% severity even in plots inoculated twice or thrice at this inoculum concentration.

The disease progress of Dactylaria leaf blight of purple nutsedge was best described by the Gompertz model (Fig. 5-1). Disease level was high in plots inoculated with 10^6 conidia/ml. Disease level increased with time until 20 days after inoculation when it started to decline in plots receiving a single application of inoculum. On the contrary, disease severity continued to increase in plots that received the second application of inoculum 14 days after the first inoculation. Disease severity levelled off after 16 days of the second inoculation (30 days after first inoculation), whereas it increased in plots that received the third inoculation (Fig. 5-1). However, the apparent

increase in disease level in these plots did not significantly increase the values of AUDPC of the plots receiving third inoculation compared to plots receiving the second inoculation. The relationships of inoculation frequency and the values of AUDPC were best described by linear regression. The 2nd order polynomial best described the relationship of inoculation frequency and the values of AUDPC of plots inoculated with 10^6 conidia/ml while the 1st order polynomial best described the relationship of the plots that received 10^5 conidia/ml (Fig. 5-2). The values of AUDPC from the plots inoculated twice or thrice did not differ greatly (Table 5-2). Disease progress was slow in plots inoculated once with 10^6 conidia/ml (r_G= 0.013) compared to plots inoculated twice (r_G = 0.140) or thrice (r_G = 0.144) (Table 5-2) with the same inoculum concentration.

The effect of inoculum concentration and inoculation frequency on the growth components of purple nutsedge is presented in Table 5-3 and in Appendix B. The growth components in plots inoculated with 10^5 conidia/ml at all inoculation frequencies were as high as in the control plots (sprayed with 0.5% Metamucil only). Only shoot dry weight and weight per tuber were reduced in plots that were inoculated one time with 10^6 conidia/ml (Table 5-3, Appendix B-1 and B-4). Nonetheless, the disease severity (AUDPC) was significantly higher in these plots compared to the control plots or the plots inoculated with 10^5 conidia/ml (Table 5-3). The minimum number of inoculations that significantly reduced the growth components of purple nutsedge was two application of 10^6 conidia/ml (Table 5-3). A third inoculation of purple nutsedge with 10^6 conidia/ml did not significantly reduce the growth components compared to the plants inoculated twice (Table 5-2, Appendix B). Reductions in the growth components as a result of two

inoculations of purple nutsedge with 10^6 conidia/ml were as follows: shoot dry weight 74%; tuber dry weight 50%; tuber number 71%; and weight per tuber 45%. Similar reductions in the growth components were recorded in plots inoculated thrice with 10^6 conidia/ml. Little or no reduction in weed growth was seen in plots inoculated once with 10^6 conidia/ml and plots inoculated thrice with 10^5 conidia. There was no significant interaction between inoculation frequency and disease severity when evaluated 15 days after inoculation. Complete eradication of purple nutsedge was not observed in any plot.

Discussion

The competitiveness of purple nutsedge with crops is promoted by its ability to grow from shoots, roots, and a combination of the shoot and the root (Marambe, 1996). Reduction of growth through one of these sources has no effect on the competitiveness of purple nutsedge as it has enormous power to recover and regenerate. This is especially true when space for growth is not limited (Gamboa, 1987). So, in order to effectively reduce the competitive effects of purple nutsedge, it is important not only to reduce nutsedge shoots, but also the roots and tubers.

Dactylaria higginsii is capable of reducing all weed-growth components of purple nutsedge, as indicated by its ability to reduce weed biomass as well as the size of the tubers. The latter are important means of propagation. However, the efficacy of this fungus to control purple nutsedge is dependent upon the conidial concentration and number of inoculations. As with other fungal bioherbicides, an inoculum concentration of 10^6 conidia/ml seems to be the effective concentration that can significantly affect the

Table 5-1. Analysis of variance of inoculum concentration and inoculation frequency of *Dactylaria higginsii* on the growth components of purple nutsedge.

Source		Shoot dry wt (g)		Tuber dry wt (g)		Tuber number		Avg. wt/tuber (g)	
	DF	MS[a]	F-value	MS	F-value	MS	F-value	MS	F-value
Sites	1	7371.31	6.14**[c]	340.70	0.28ns[b]	22810.26	2.56ns	0.014	3.19ns
Block (Sites)	6	1186.88	0.99ns	6208.00	0.11ns	12039.43	0.23ns	0.003	0.61ns
Inoc. conc.	2	247853.27	206.31***[d]	1629817.96	146.98***	3832404.80	214.61***	0.583	130.58***
Inocul. freq.	2	110945.27	92.35***	744966.65	67.18***	1407732.42	78.96***	0.19	42.73***
Inoc. conc. x Inoc. freq	2	114109.14	94.98***	1027125.99	92.63***	1512853.11	84.86***	0.14	31.65***

[a]MS = Mean Square.
[b]ns = Not significant.
[c]** = Significant at $P < 0.01$.
[d]*** = Significant at $P < 0.001$.
Inoc. conc. = Inoculum concentration.
Inoc. freq. = Inoculation frequency.

Table 5-2. Effect of inoculum concentration and inoculation frequency on disease severity expressed as the area under the disease progress curve (AUDPC) and the apparent infection rate (r_G) of Dactylaria leaf blight of purple nutsedge caused by *Dactylaria higginsii*.

Inoc. conc. (conidia/ml)	Inoculation frequency	AUDPC[x]	r_G[y]
Control	0	0	0
10^5	1	162.875	NR
10^5	2	233.500	NR
10^5	3	277.688	NR
10^6	1	785.625	0.013
10^6	2	2744.375	0.140
10^6	3	2779.500	0.144

[x]Area under the disease progress curve (AUDPC) was calculated from disease severity ratings from the means of two trials, each with four replicates.
[Y]Apparent infection rate was calculated by using the Gompertz model.

Table 5-3. The effect of inoculum concentration and inoculation frequency on the growth components of purple nutsedge inoculated with *Dactylaria higginsii*.

Inoculum concentration	Inoculation frequency	Shoot dry wt (g)	Tuber dry wt (g)	Tuber number	Weight/tuber
Control	0	308.39	1118.71	821.88	0.73
10^5	1	295.20	1091.11	814.02	0.74
10^5	2	299.33	1135.41	827.90	0.73
10^5	3	294.32	1138.32	819.79	0.72
10^6	1	288.77	1133.69	767.20	0.68
10^6	2	91.76	61.13	264.47	0.45
10^6	3	78.31	63.69	231.83	0.41
			Contrast		
Control vs 10^5		ns	ns	ns	ns
Control vs 10^6		***	***	***	***
10^5 vs 10^6		***	***	***	***

ns = Not significant.
***$P< 0.001$.
All values are averages of two trials with four replicates.

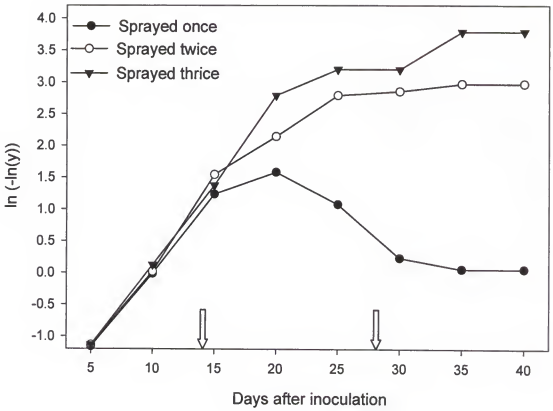

Fig. 5-1. Disease progress curves of Dactylaria leaf blight on purple nutsedge caused by *Dactylaria higginsii*. Each data point is the transformed value of disease severity rating and represents the mean of two trials, each with four replicates using the Gompertz model. The arrows indicate the days when the second and third applications were made.

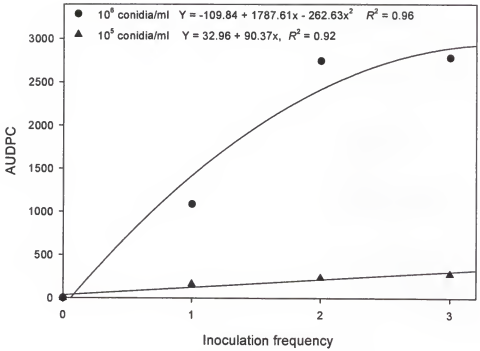

Fig. 5-2. Relationship between inoculation frequency and disease severity (in term of area under the disease progress curve) of purple nutsedge inoculated with of *Dactylaria higginsii*. Each data point is the mean of two trials, each with four replicates.

growth of purple nutsedge (Makowski, 1993; Morin et al.,1989; Ormeno-Nunez et al., 1988; Wymore and Watson, 1989). However, a higher inoculum concentration (> 10^6 conidia/ml) should be used to give better and consistent control in the field. A higher concentration (> 10^6 conidia/ml) was recommended by Morin et al. (1989) for effective control of bindweed (*Convolvulus arvensis* L.) with *Phomopsis convolvulus* Ormeno, especially since the efficiency of the inoculum of this fungus is influenced by environmental factors. When applied at less than 10^6 conidia/ml, *D. higginsii* did not reduce the growth components of purple nutsedge, even though a significant level of control was achieved with 10^5 conidia/ml in greenhouse trials. An inoculum concentration of 10^5 conidia/ml or 10^6 conidia/ml applied once was also not sufficient to curtail the lush growth of purple nutsedge. Since the level of inoculum was low to start with, the level of secondary infection was also low, possibly due to nonconducive environmental factors that prevent the build-up of secondary inoculum. Purple nutsedge, which can recover and regenerate rapidly from previously low levels of infection, is capable of escaping secondary infection and out-growing the low disease levels.

Spread of secondary inoculum has been reported to be common in plots sprayed with *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene*, a bioherbicide used to control northern jointvetch (*Aeschynomene virginica* L. (B.S.P.)) (TeBeest and Templeton, 1985), with *Alternaria macrospora* Zimm., a pathogen of spurred anoda (*Anoda cristata* [L.] Schlecht.) (Walker, 1981), and with *Colletotrichum coccodes* (Wallr.) Hughes, a pathogen of velvetleaf *(Abutilon theophrasti* Medik.) (Wymore and Watson, 1989). There was only limited secondary spread of *D. higginsii* in the field in

these trials, possibly due to unfavorable temperature. The average maximum temperature during the month of July and August 1996, when these studies were done, was 32°C (Appendix C-1). This temperature is above the temperature range conducive for maximum disease development (20°C to 30°C, Chapter 3).

Unlike other weeds, where single application of bioherbicides at effective concentration is enough to achieve good control in the field (e.g., Collego; TeBeest and Templeton,1985), single application of *D. higginsii* did not significantly reduce the growth components of purple nutsedge. Also one application of inoculum at the most effective dose (10^6 conidia/ml or 10^{12} conidia in 1000 liters/ha) was not sufficient to control this weed, because once the shoot died, the plants regenerated from the tubers and the available inoculum was apparently not sufficient to infect the subsequent growth.

Regular applications of certain bioherbicides have been reported to provide long-term control of the target weed. For example, Imaizumi et al. (1997) reported that Camperico® (*Xanthomonas campestris* pv. *poae*) provided excellent long-term control of annual bluegrass (*Poa annua* L.) when it was applied regularly. This is true with *Dactylaria higginsii*. Multiple inoculations of *D. higginsii* at 10^6 conidia or higher are required to effectively reduce purple nutsedge growth in the field.

Inability to control purple nutsedge with one-time application of control measures is also known in the case of chemical herbicides and physical removal of the shoots. Zandstra et al. (1974) and Amerasinghe (1992) found that glyphosate did not effectively control this weed when applied as a single application due to poor translocation of the herbicide to the tubers. Komai and Ueki (1982) and Santos et al. (1997) reported that

shoot emergence and tuber formation in purple nutsedge were reduced significantly when the plants were cut three or more times. They attributed the reduction in tuberization to the utilization of starch for the shoot regrowth after cutting. Marambe (1996), who was working on the effect of defoliation on starch metabolism, reported that repeated defoliation significantly reduced regrowth and starch content of purple nutsedge tubers when compared to single or partial defoliation and nondefoliated control. Defoliation treatment did not have any significant suppressing effect on the regrowth of nutsedge tubers since the initial loss of leaf area was compensated by the rapid reproduction of new shoots. Holt et al. (1967) attributed the reduction in tuber regeneration to the depletion of food reserves when purple nutsedge plants were treated with several applications of herbicides. This explanation was later proved to be correct by Komai and Ueki (1982) and Marambe (1996) who reported that degradation of starch in purple nutsedge tuber was positively correlated with endoamylase activity. This enzyme was postulated by these authors as the major factor that determines the ability of purple nutsedge to regenerate.

It is clear from this study that *D. higginsii* is a highly promising bioherbicidal agent for the control of purple nutsedge. It is also evident that the extraordinary ability of purple nutsedge to regenerate could be substantially suppressed, and the slow build-up of secondary inoculum could be overcome by multiple sprayings of *D. higginsii*. A minimum of two sprayings is required to effectively reduce the competitiveness of purple nutsedge. Further work is therefore justified to develop and register *D. higginsii* as a bioherbicide for purple nutsedge.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Purple nutsedge has been called the world's worst weed. Despite various attempts to control it, this weed continues to increase in severity under the current cropping systems (Gricher et al., 1992). Various management strategies are available to control purple nutsedge, but most of these are not entirely satisfactory when used alone because each has its limitations (Doll, 1986; Glaze, 1987). Among the major reasons for the difficulty in controlling this weed are its ability for rapid growth and proliferation from the rhizomes and tubers and the production of dormant tubers. The dormant tubers act as a weak sink to herbicides (Zandstra and Nishimoto, 1977). Since the tubers play a central role in the establishment of new shoots and underground organs, any measure that can reduce and inhibit tuberization holds great promise for the control of purple nutsedge.

The use of biological control agents is an alternative or a complementary tactic to reduce herbicide inputs. Charudattan (1991) comprehensively reviewed the bioherbicide research worldwide and reported that this strategy is receiving increasing attention throughout the world. Research into the use of biological control of purple nutsedge relied first on the use of nutsedge insects, but it failed to progress due to lack of efficacy of the insects in the field. Attempts to use fungal pathogens to control this weed have only recently begun. The search for bioherbicides to control this weed has been facilitated by the absence of effective chemical herbicides and the imminent banning of

methyl bromide from agricultural use in the U.S. by the year 2000. This broad-spectrum soil fumigant has been the mainstay to control this weed in high-value crops in regions such as California and Florida.

Even though there are several fungal pathogens reported as potential bioherbicide candidates, only a few have been proven to be legitimate candidates in both greenhouse and field trials. A fungus, *Dactylaria higginsii* (Luttrell) M.B. Ellis, isolated from diseased purple nutsedge plants, was shown to be among the few pathogens proven to be highly pathogenic to purple nutsedge (Kadir and Charudattan, 1996). The potential of this fungus as a bioherbicide for the control of purple nutsedge has been demonstrated both in the greenhouse and in the field (Kadir et al., 1997a; 1977b).

The delimitation of host range is an important component of biological control programs. The host specificity of the candidate agents must be determined to assure its safety to desirable plant species. Generally, for indigenous plant pathogens, strict host specificity is not required. Nonetheless, the pathogen must be demonstrated to have an acceptable level of safety (Wasphere, 1982; Watson, 1985). The rationale here is that the biocontrol agent is already present in the environment where it will be used, the nontarget plants have already been exposed to the pathogen, and therefore any adverse effect of the agent on nontarget plants would have become evident (Leonard, 1982; Watson, 1990).

Wasphere (1974) proposed a phylogenetic scheme of testing to assure the safety of biocontrol agents to nontarget plants. This approach has received wide acceptance in biological weed control research. Accordingly, in this study, the phylogenetic scheme was used to select test plants, and the host range of *D. higginsii* was found to be restricted to the genus *Cyperus* only. Important crop plants and other economic plants tested were

immune. However, histopathological studies are needed to examine possible latent infections on nonhost plants and to confirm the specificity and safety of this fungus.

Typically, low initial inoculum level contributes to the failure of disease epidemics to develop and persist in weed populations (Watson and Wymore, 1990). In this study, by increasing the initial inoculum level of *D. higginsii* through inundative application it was possible to increase the level of control of purple nutsedge. Thus, it was shown that this potential bioherbicide pathogen could cause high levels of epidemics through manipulation of the initial inoculum concentration. Under controlled environments, high levels of purple nutsedge control were achieved when *D. higginsii* was applied at the rate of 100 ml/m^2 with an inoculum concentration of 10^6 conidia per milliliter or the equivalent of 10^{12} conidia per 1000 liter per ha. This volume of the spray suspension may not be a feasible measurement for field use. Therefore, a study should be initiated to determine if low and ultra low volume spray suspensions with increased spore concentration, could help distribute spores uniformly on purple nutsedge plants and produce a high level of control.

Plant age is considered a factor affecting disease expression and plant susceptibility. Therefore, the determination of the plant-growth stages at which the host is susceptible to a bioherbicide is important for understanding the potential of a bioherbicide candidate (Watson and Wymore, 1990). Hence, through a greenhouse experiment it was determined that *D. higginsii* was highly pathogenic to younger plants (4- to 6-leaf stage compared to > 6-leaf stage) at a temperature range from 20°C to 30°C and with as little as 12 h of exposure to dew period (100% relative humidity). At this temperature range and dew period, *D. higginsii* was capable of providing excellent

control of young purple nutsedge plants. This temperature and dew-period requirement coincide with the conditions common during the early period of the cropping season in the warm temperate regions of the world. Additionally, the most susceptible growth stage (4- to 6-leaf stage) was well within the time when weed-control measures, such as conventional postemergence chemical herbicides are applied. This finding demonstrated that inoculum density, plant-growth stage, and dew-period temperature do not constitute biological constraints to *D. higginsii* as a bioherbicide. However, the dew-period duration is likely to be a limitation if this bioherbicide is to be applied in the tropical regions of the world. Nonetheless, it appears possible to overcome this dew-period constraint with the use of suitable amendments to improve inoculum survival and infectivity. In this study, it was possible to use Metamucil®or N-Gel® to bypass the dew requirement and cause high levels of disease under greenhouse conditions. Other types of formulations, such as invert emulsions, crop-oil suspensions, and other surfactants should be evaluated. This study will help determine the most effective formulation for delivering the inoculum of this bioherbicide.

The ability of *D. higginsii* to suppress the growth of purple nutsedge plants when grown in competition with a crop plant was determined in the greenhouse using tomato and pepper as crops. This study demonstrated that *D. higginsii*, when applied at 10^6 conidia/ml under the cropping situation, was capable of reducing purple nutsedge growth components by almost 90%. This translated into an effective suppression of purple nutsedge interference. The ability of *D. higginsii* to suppress purple nutsedge competition with crops remains to be validated in the field to determine its applicability to commercial agriculture.

The efficacy of *D. higginsii* in purple nutsedge-infested, crop-free fields has been demonstrated. Multiple applications of *D. higginsii* at an inoculum dose of 10^6 conidia per milliliter at the rate of 100 ml per m^2 (equivalent of 10^{12} conidia per 1000 liter per ha) was required to effectively suppress the growth of purple nutsedge. An inoculum concentrations lower than this level did not provide effective control. The high conidial concentration required for effective control of purple nutsedge may become a limiting factor if inoculum production is difficult. However, this is not the case with *D. higginsii* since this fungus is capable of growing and producing abundant of conidia on various media.

The ability of *D. higginsii* to effectively control purple nutsedge suggested the beneficial potential of this fungus as a bioherbicide. Since chemical herbicides are not likely to be effective in controlling this weed in every cropping situation, this fungus appears to offer an alternative method to control purple nutsedge. Further studies are needed to develop methods of inoculum production and to identify additives that prolong inoculum viability. Further studies are required to confirm the efficacy of this bioherbicide in commercial fields. Finally, attempts should be made to register *D. higginsii* for commercial use.

APPENDIX A
THE EFFECT OF THE INTERACTION OF INOCULUM CONCENTRATION AND INOCULATION FREQUENY OF *Dactylaria higginsii* ON THE INTERFERENCE OF PURPLE NUTSEDGE WITH PEPPER AND TOMATO

Table A-1. Regression equations that describe the relationship of the effect of *Dactylaria higginsii* on the yields of pepper and tomato planted at different initial tuber densities of purple nutsedge.

Treatment	Regression equation	R^2
	Pepper	
Control	$Y = -0.22 + 0.75X - 0.02X^2$	0.93
10^4 conidia/ml	$Y = -2.38 + 0.74X - 0.02X^2$	0.96
10^6 conidia/ml	$Y = 0.88 + 0.06X - 0.002X^2$	0.63
	Tomato	
Control	$Y = -3.45 + 0.52X - 0.0001X^2$	0.88
10^4 conidia/ml	$Y = -2.99 + 0.56X - 0.01X^2$	0.84
10^6 conidia/ml	$Y = 1.14 + 0.21X - 0.001X^2$	0.23

Table A-2. Regression equations that describe the relationship of the effect of *Dactylaria higginsii* on the shoot dry weight of purple nutsedge grown in the presence of pepper and tomato.

Treatment	Regression equation	R^2
	Pepper	
Control	$Y = 1.73 + 1.07X - 0.02X^2$	0.95
10^4 conidia/ml	$Y = 3.59 + 1.11X - 0.02X^2$	0.92
10^6 conidia/ml	$Y = 0.20 + 0.05X - 0.001X^2$	0.99
	Tomato	
Control	$Y = 1.37 + 1.43X - 0.03X^2$	0.96
10^4 conidia/ml	$Y = 9.30 + 1.35X - 0.03X^2$	0.95
10^6 conidia/ml	$Y = 0.51 + 0.09X - 0.002X^2$	0.90

Table A-3. Regression equations that describe the relationship of the effect of *Dactylaria higginsii* on the tuber dry weight of purple nutsedge grown in the presence of pepper and tomato.

Treatment	Regression equation	R^2
	Pepper	
Control	$Y = 5.11 + 1.20X - 0.003X^2$	0.93
10^4 conidia/ml	$Y = 7.22 + 1.14X - 0.002X^2$	0.92
10^6 conidia/ml	$Y = 0.62 + 0.06X - 0.0001X^2$	0.96
	Tomato	
Control	$Y = 3.80 + 1.11X - 0.02X^2$	0.91
10^4 conidia/ml	$Y = 4.10 + 1.09X - 0.02X^2$	0.94
10^6 conidia/ml	$Y = 0.11 + 0.03X - 0.0001X^2$	0.96

Table A-4. Regression equations that describe the relationship of the effect of *Dactylaria higginsii* on the final tuber numbers of purple nutsedge grown in the presence of pepper and tomato.

Treatment	Regression equation	R^2
	Pepper	
Control	$Y = 15.23 + 2.7X - 0.06X^2$	0.90
10^4 conidia/ml	$Y = 15.17 + 2.52X - 0.05X^2$	0.92
10^6 conidia/ml	$Y = 1.53 + 0.05X - 0.001X^2$	0.65
	Tomato	
Control	$Y = 13.22 + 2.37X - 0.005X^2$	0.91
10^4 conidia/ml	$Y = 16.45 + 2.19X - 0.005X^2$	0.95
10^6 conidia/ml	$Y = 0.46 + 0.12X - 0.0002X^2$	0.98

APPENDIX B
CONTRASTS FOR THE MIXED MODEL OF PURPLE NUTSEDGE DENSITIES AND THE INTERACTION OF INOCULATION FREQUENCY AND INOCULUM CONCENTRATION OF *Dactylaria higginsii*

Table B-1. Orthogonal contrasts for the effect of inoculum concentration of *Dactylaria higginsii* on the shoot dry weight of purple nutsedge.

	Inoculation frequency		Differences	*t*-value
0 vs 10^5	1	1	13.19	1.20ns
0 vs 10^5	1	2	9.06	0.79ns
0 vs 10^5	1	3	13.88	1.24ns
0 vs 10^6	1	1	19.62	1.75ns
0 vs 10^6	1	2	216.63	19.37***
0 vs 10^6	1	3	230.08	20.57***
10^5 vs 10^5	1	2	-4.13	-0.33ns
10^5 vs 10^5	1	3	0.69	0.06ns
10^5 vs 10^6	1	1	6.43	0.53ns
10^5 vs 10^6	1	2	203.44	16.84***
10^5 vs 10^6	1	3	216.89	17.95***
10^5 vs 10^5	2	3	4.82	0.39ns
10^5 vs 10^6	2	1	10.56	0.85ns
10^5 vs 10^6	2	2	207.57	16.65***
10^5 vs 10^6	2	3	221.02	17.22***
10^5 vs 10^6	3	1	5.74	0.47ns
10^5 vs 10^6	3	2	202.75	16.55***
10^5 vs 10^6	3	3	216.20	17.64***
10^6 vs 10^6	1	2	197.01	16.08***
10^6 vs 10^6	1	3	210.46	17.17***
10^6 vs 10^6	2	3	13.45	1.10ns

ns = Not significant.
*** Significant at $P < 0.001$.

Table B-2. Orthogonal contrasts for the effect of inoculum concentration of *Dactylaria higginsii* on the tuber dry weight of purple nutsedge.

	Inoculation frequency		Differences	*t*-value
0 vs 10^5	1	1	27.60	0.83ns
0 vs 10^5	1	2	-16.70	- 0.48ns
0 vs 10^5	1	3	-19.60	-0.58ns
0 vs 10^6	1	1	-14.98	-0.44ns
0 vs 10^6	1	2	557.58	16.41***
0 vs 10^6	1	3	555.02	16.33***
10^5 vs 10^5	1	2	-44.30	-1.18ns
10^5 vs 10^5	1	3	-47.20	-1.29ns
10^5 vs 10^6	1	1	-42.58	-1.16ns
10^5 vs 10^6	1	2	529.98	14.44***
10^5 vs 10^6	1	3	527.42	14.37***
10^5 vs 10^5	2	3	-2.90	-0.08ns
10^5 vs 10^6	2	1	1.72	0.05ns
10^5 vs 10^6	2	2	574.29	15.16***
10^5 vs 10^6	2	3	571.72	15.09***
10^5 vs 10^6	3	1	4.63	0.12ns
10^5 vs 10^6	3	2	577.19	15.50***
10^5 vs 10^6	3	3	574.63	15.43***
10^6 vs 10^6	1	2	572.56	15.38***
10^6 vs 10^6	1	3	570.00	15.31***
10^6 vs 10^6	2	3	-2.56	-0.07ns

ns = Not significant.
*** Significant at $P < 0.001$.

Table B-3. Orthogonal contrasts for the effect of inoculum concentration of *Dactylaria higginsii* on the final tuber number of purple nutsedge.

	Inoculation frequency		Differences	*t*-value
0 vs 10^5	1	1	7.86	0.26ns
0 vs 10^5	1	2	-6.01	-0.19ns
0 vs 10^5	1	3	2.09	0.07ns
0 vs 10^6	1	1	54.68	1.97ns
0 vs 10^6	1	2	557.41	18.29***
0 vs 10^6	1	3	590.05	19.36***
10^5 vs 10^5	1	2	-13.88	-0.41ns
10^5 vs 10^5	1	3	-5.78	-0.18ns
10^5 vs 10^6	1	1	46.82	1.42ns
10^5 vs 10^6	1	2	549.55	16.70***
10^5 vs 10^6	1	3	582.18	17.69***
10^5 vs 10^5	2	3	8.10	0.24ns
10^5 vs 10^6	2	1	60.70	1.79ns
10^5 vs 10^6	2	2	563.43	16.59***
10^5 vs 10^6	2	3	596.06	17.55***
10^5 vs 10^6	3	1	52.60	1.58ns
10^5 vs 10^6	3	2	555.32	16.64***
10^5 vs 10^6	3	3	587.96	17.61***
10^6 vs 10^6	1	2	502.73	15.06***
10^6 vs 10^6	1	3	535.36	16.04***
10^6 vs 10^6	2	3	32.64	0.98ns

ns = Not significant.
*** Significant at $P < 0.001$.

Table B-4. Orthogonal contrasts for the effect of inoculum concentration of *Dactylaria higginsii* on the average dry weight per tuber of purple nutsedge.

	Inoculation frequency		Differences	*t*-value
0 vs 10^5	1	1	-0.01	-0.57ns
0 vs 10^5	1	2	0.01	0.05ns
0 vs 10^5	1	3	0.01	0.46ns
0 vs 10^6	1	1	0.06	2.62**
0 vs 10^6	1	2	0.28	13.11***
0 vs 10^6	1	3	0.32	14.84***
10^5 vs 10^5	1	2	0.01	0.55ns
10^5 vs 10^5	1	3	0.02	0.95ns
10^5 vs 10^6	1	1	0.07	2.94**
10^5 vs 10^6	1	2	0.29	12.65***
10^5 vs 10^6	1	3	0.33	14.26***
10^5 vs 10^5	2	3	0.01	0.37ns
10^5 vs 10^6	2	1	0.06	2.31*
10^5 vs 10^6	2	2	0.02	11.71***
10^5 vs 10^6	2	3	0.32	13.27***
10^5 vs 10^6	3	1	0.05	1.97ns
10^5 vs 10^6	3	2	0.27	11.54***
10^5 vs 10^6	3	3	0.31	13.13***
10^6 vs 10^6	1	2	0.23	9.57***
10^6 vs 10^6	1	3	0.26	11.16***
10^6 vs 10^6	2	3	0.04	1.59ns

ns = Not significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

APPENDIX C
WEATHER DATA OF THE TRIAL SITES

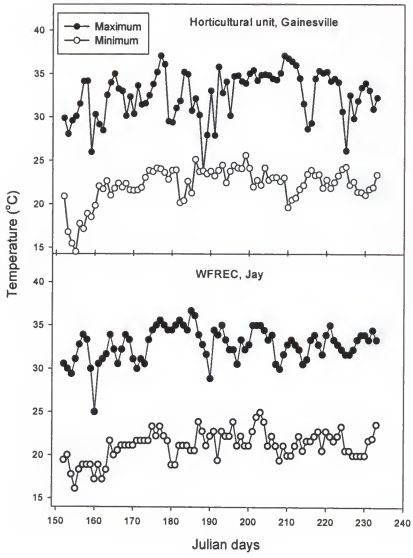

Fig. C-1. Average minimum and maximum daily temperature (°C) for the months of June, July, and August, 1996 for the Horticultural Unit Experimental Station and the West Florida Research and Education Center (WFREC), Jay, FL. (Data obtained from the Dept. of Agricultural Engineering, University of Florida, Gainesville, FL and from Dr. Brecke, WFREC, Jay, FL).

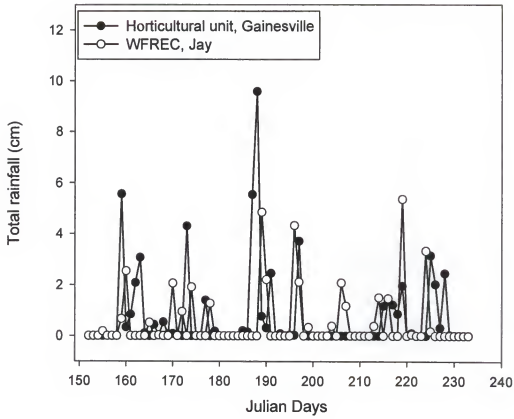

Fig. C-2. Total daily rainfall (cm) for the months of June, July, and August, 1996 for the Horticultural Unit Experimental Station and the West Florida Research and Education Center (WFREC), Jay, FL. (Data obtained from the Dept. of Agricultural Engineering, University of Florida, Gainesville, FL and from Dr. Brecke, WFREC, Jay, FL).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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